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(54) Title: IMPROVED ISOPRENOID PRODUCTION

(57) Abstract: Isolated polynucleotides encoding polypeptides having the activity of enzymes in the mevalonate pathway, e.g. hydroxymethylglutaryl-CoA reductase, isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase, mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; are provided, useful for recombinantly producing isoprenoid compounds such as carotenoids like phytoene, lycopene,  $\beta$ -carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone and adonirubin. Expression vectors, cultured cells, and methods of making isoprenoid compounds are also provided.

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### Improved Isoprenoid Production

The present invention relates to novel polynucleotides and polypeptide sequences useful in the isoprenoid biosynthetic pathway. More particularly, the present invention provides recombinantly produced cells that exhibit improved production of zeaxanthin. Methods of making and using such cell lines are also provided.

Carotenoids are commercially important C-40 isoprenoid compounds used as nutritional supplements, pharmaceuticals and food colorants for humans and as pigments for animal feed. Currently industrially important carotenoids are produced mainly by chemical synthesis ( $\beta$ -carotene, canthaxanthin and astaxanthin) or extraction from natural sources (lutein from marigold, capsanthin from paprika). Production of carotenoids, however, using microorganisms has been achieved in some cases. For example,  $\beta$ -carotene is produced by fermentation with the fungus *Blakeslea trispora* (US 5,328,845) or by pond culture using the halotolerant alga *Dunaliella salina* [Borowitzka, J. Biotechnol. 70:313-321 (1999)]. Lycopene production has also been reported in *B. trispora* (WO 00/77234).

Astaxanthin is produced by fermentation using yeast (*Phaffia rhodozyma*, (recently renamed *Xanthophyllomyces dendrorous*)) (US 6,015,684) or in photobioreactors or open ponds using the alga *Haematococcus pluvialis* [Lorenz and Cysewski, Trends Biotechnol. 18:160-167 (1999); Olaizola, J. Appl. Phycol. 12:499-506 (2000)]. Such microbial production systems, however, do not produce carotenoids in amounts sufficient for economical industrial scale production.

In the mid-1960's, scientists at Hoffmann-La Roche isolated several marine bacteria that produced the yellow carotenoid zeaxanthin, which has application in poultry pigmentation and in the prevention of age-related macular degeneration in humans. One bacterium, which showed promising levels of zeaxanthin production, was given the strain designation R-1512, and it was deposited at the American Type Culture Collection (ATCC, Manassas, VA, USA) as strain ATCC 21588 (US 3,891,504). Using the accepted taxonomic standards of that time (classification performed by the Eidgenössische Technische Hochschule (Zürich) and the National Collection of Industrial Bacteria, Torry Research Station (Aberdeen, Scotland)), the zeaxanthin-producing organism was classified as a member of the genus *Flavobacterium*, but no species designation was assigned.

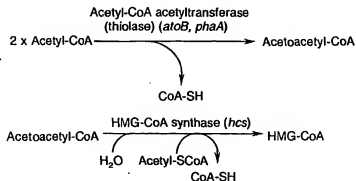
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An extensive mutagenesis and screening program was subsequently conducted to isolate mutants of R-1512 with higher zeaxanthin productivities. With respect to the presently described work, two such mutants are significant. These mutants, listed in order of their zeaxanthin productivities, are R1534 and R114. A variety of other mutants have been used over the years for biochemical studies of carotenoid biosynthesis [Goodwin, *Biochem. Soc. Symp.* 35:233-244 (1972); McDermott et al., *Biochem. J.* 134:1115-1117 (1973); Britton et al., *Arch. Microbiol.* 113:33-37 (1977); Mohanty et al., *Helvetica Chimica Acta* 83:2036-2053 (2000)].

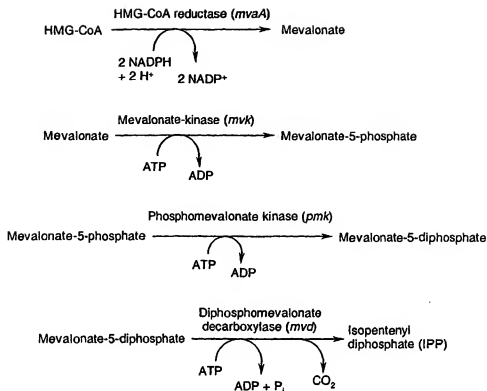
The early attempts to develop a commercially viable fermentation process for the production of zeaxanthin using classically derived mutants of strain R-1512 were not successful. However, with the advent of molecular biology, the possibility arose that higher zeaxanthin-producing strains could be developed. The first step in this direction was taken with the cloning and sequencing of the carotenoid gene cluster from strain R1534 (US 6,087,152), which is hereby incorporated by reference as if recited in full herein). US 6,087,152 discloses that the carotenoid genes were functionally expressed in *Escherichia coli* and *Bacillus subtilis* resulting in zeaxanthin production in these hosts. US 6,087,152 also disclosed that by modifying the carotenoid gene cluster or by adding a gene from an astaxanthin producing bacterium, it was possible to produce carotenoids other than zeaxanthin (EP 872,554). Moreover, EP 872,554 disclosed that carotenoid production was increased in strain R1534 by introducing cloned carotenoid gene clusters on a multi-copy plasmid.

Despite the enormous structural diversity in isoprenoid compounds, all are biosynthesized from a common C-5 precursor, isopentenyl pyrophosphate (IPP). Up until the early 1990's it was generally accepted that IPP was synthesized in all organisms via the mevalonate pathway, even though some experimental results were not consistent with this biogenic scheme [Eisenreich et al., *Chemistry and Biology* 5:R221-R233 (1998)].

Mevalonate pathway:

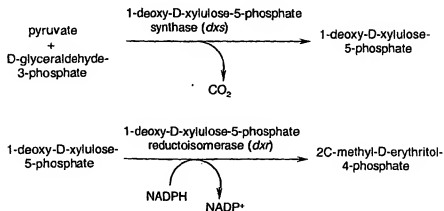


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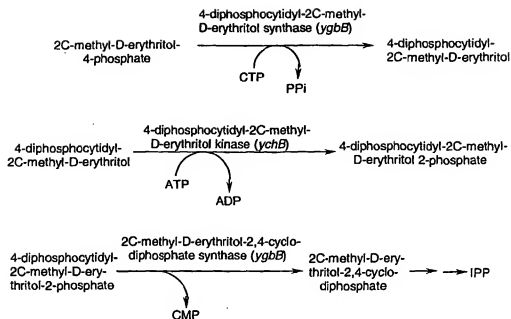


- 5 The discrepancies have since been reconciled by the discovery of an alternate pathway of IPP biosynthesis, the deoxyxylulose (DXP) pathway (Note: The alternate pathway of IPP biosynthesis has been referred to by various names in the scientific literature (DXP pathway, DOXP pathway, MEP pathway, GAP/pyruvate pathway and the non-mevalonate pathway). We use the name DXP pathway here only for the sake of simplicity). The first
- 10 five reactions of the DXP pathway have been identified [Herz et al., Proc. Nat. Acad. Sci. 97:2486-2490 (2000)], but the subsequent steps leading to formation of IPP have not yet been elucidated.

DXP pathway:



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- McDermott et al. (supra) and Britton et al. [J. Chem. Soc. Chem. Comm. p. 27 (1979)]
- 5 showed that crude extracts of zeaxanthin producing mutant strains derived from the original Roche isolates incorporated labeled mevalonate into zeaxanthin. While there was no reason to question this evidence for IPP biosynthesis via the mevalonate pathway, the work was done prior to the discovery of the DXPP pathway, and it has been reported that some bacteria (*Streptomyces* species) possess both pathways for IPP synthesis and that expression
- 10 of these pathways is temporally regulated [Seto et al., Tetrahedron Lett. 37:7979-7982 (1996); Dairi et al., Mol. Gen. Genet. 262:957-964 (2000)]. In addition, at present, only a small number of eubacteria have been shown to possess the mevalonate pathway for IPP synthesis. The genes encoding the enzymes of the mevalonate pathway have been cloned and sequenced from some of these bacteria [Wilding et al., J. Bacteriol. 182:4319-4327
- 15 (2000); Takagi et al., J. Bacteriol. 182:4153-4157 (2000)].

Several examples exist where the application of metabolic engineering has succeeded in altering or improving carotenoid production in microorganisms [Lagarde et al., Appl. Env. Microbiol. 66:64-72 (2000); Wang et al., Biotechnol. Bioeng. 62:235-241 (1999); Wang et al., Biotechnol. Prog. 16:922-926 (2000) (and references therein); Sandmann et al., Trends

20 Biotechnol. 17:233-237 (2000); Misawa and Shimada, J. Biotechnol. 59:169-181 (1998); Matthews and Wurtzel, Appl. Microbiol. Biotechnol. 53:396-400 (2000); Albrecht et al., Nature Biotechnol. 18:843-846 (2000); Schmidt-Dannert et al., Nature Biotechnol. 18:750-753 (2000)]. For example, *E. coli*, a non-carotenogenic bacterium, can be engineered to produce carotenoids by introducing the cloned carotenoid (*crt*) genes from the bacteria

*Agrobacterium aurantiacum*, *Erwinia herbicola* or *Erwinia uredovora* (Misawa and Shimada, supra). Harker and Bramley [FEBS Lett. 448:115-119 (1999)] and Matthews and Wurtzel (supra) disclosed that carotenoid production in such engineered *E. coli* strains could be increased by over-expressing the gene coding for 1-deoxy-D-xylulose 5-phosphate

5 synthase (DXPS), the first enzyme in the DXP pathway (*E. coli* possesses only the DXP pathway for isoprenoid biosynthesis and does not use the mevalonate pathway [Lange et al., Proc. Nat. Acad. Sci. 97:13172-13177 (2000)]). Harker and Bramley (supra) also disclosed an increase in the isoprenoid compound ubiquinone-8, in the cells overproducing DXPS. These results supported the hypothesis that limited availability of IPP, resulting

10 from insufficient *in vivo* activity of DXPS, was limiting the production of carotenoids and other isoprenoid compounds in the engineered strains. Using a similar *E. coli* system, Kim and Keasling [Biotechnol. Bioeng. 72:408-415 (2001)] disclosed that the combined over-expression of the genes encoding DXPS and the second enzyme of the DXP pathway, DXP reductoisomerase (1-deoxy-D-xylulose-5-phosphate reductoisomerase) gave higher carotenoid production than over-expression of just the gene encoding DXPS.

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All of these studies were done in *E. coli* engineered to produce carotenoids. Accordingly, one disadvantage to these studies was that the amount of carotenoids produced by these recombinant *E. coli* strains were very low compared to the amounts produced by even

20 non-recombinant microorganisms used for industrial production of carotenoids. Furthermore, improved carotenoid production in bacteria by genetic engineering of the IPP biosynthetic pathway has only been shown in organisms that utilize the DXP pathway for IPP formation. No similar studies have been reported for bacteria that produce IPP via the mevalonate pathway.

Metabolic engineering of the mevalonate pathway to improve production of isoprenoid compounds has been reported in yeast. For example, WO 00/01649 disclosed that production of isoprenoid compounds is increased in *Saccharomyces cerevisiae* when the gene coding for 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) is over-expressed. However, it has not been shown that this strategy improves isoprenoid production in bacteria, and in particular, it has not been shown that carotenoid

30 production in bacteria can be improved by amplifying expression of mevalonate pathway genes. While it has been shown that some mevalonate pathway genes from eukaryotes [Campos et al., Biochem. J. 353:59-67 (2001)] and from the bacterium *Streptomyces* sp. strain CL190 (Takagi et al., supra) can be expressed in *E. coli*, no increase in isoprenoid production was reported in the strains.

In addition to the reactions that form IPP (via the DXP or mevalonate pathways) and the reactions that convert farnesyl pyrophosphate (FPP) to various other isoprenoids (e.g., carotenoids, quinones) two other reactions are known to be involved in isoprenoid biosynthesis. IPP isomerase interconverts IPP and its isomer, dimethylallyl pyrophosphate (DMAPP). Two forms of IPP isomerase exist, the type 1 enzyme is well known in eukaryotes and some bacteria, and the newly identified type 2 enzyme that is FMN- and NADP(H)-dependent [Kaneda et al., Proc. Nat. Acad. Sci. 98:932-937 (2001)].

Several reports disclose that in *E. coli* engineered to produce carotenoids, amplification of native or heterologous type 1 IPP isomerase (*idi*) genes stimulates carotenoid production [Kajiura et al., Biochem. J. 324:421-426 (1997); Verdoes and van Ooyen, Acta Bot. Gallica 146:43-53 (1999); Wang et al., supra]. In one report (Wang et al, supra), it was further disclosed that over-expression of the *ispA* gene, encoding FPP synthase (Farnesyl diphosphate synthase) increased carotenoid production in an engineered carotenogenic strain of *E. coli* when combined with over-expression of the *idi* and *crtE* (GGPP synthase/Geranylgeranyl diphosphate synthase) genes. As is the case for the pathway of IPP biosynthesis, however, it has not been shown that over-expression of genes coding for IPP isomerase or FPP synthase improves carotenoid production in a naturally carotenogenic microorganism. Also, the levels of carotenoids produced in the *E. coli* strains described above are very low, and it has not been shown that these strategies work in an industrial microorganism where carotenoid production was already high.

In sum, there is no prior evidence that increased expression of gene(s) coding for enzymes of the mevalonate pathway can improve production of carotenoids in naturally carotenogenic bacteria or in naturally non-carotenogenic bacteria engineered to be carotenogenic.

One embodiment of the present invention is an isolated polypeptide that includes an amino acid sequence selected from the following group: (a) an amino acid sequence shown as residues 1 to 340 of SEQ ID NO:43; (b) an amino acid sequence shown as residues 1 to 349 of SEQ ID NO:45; (c) an amino acid sequence shown as residues 1 to 388 of SEQ ID NO:47; (d) an amino acid sequence shown as residues 1 to 378 of SEQ ID NO:49; (e) an amino acid sequence shown as residues 1 to 305 of SEQ ID NO:51; (f) an amino acid sequence shown as residues 1 to 332 of SEQ ID NO:53; (g) a fragment of an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, wherein said fragment has at least 30 contiguous amino acid residues; (h) an amino acid sequence of a fragment of a polypeptide selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, the fragment having the activity of HMG-CoA reductase, isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase (HMG-CoA

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synthase), mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; (i) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides of SEQ ID NO:42 or a complement of SEQ ID NO:42, wherein the  
5 polypeptide has the activity of HMG-CoA reductase, isopentenyl diphosphate isomerase, HMG-CoA synthase, isopentenyl diphosphate isomerase, mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; and (j) a conservatively modified variant of SEQ ID NOs:43, 45, 47, 49, 51 or 53.

As noted above, the present invention includes SEQ ID Nos: 43, 45, 47, 49, 51, and 53,  
10 which are polypeptide sequences that correspond to the following enzymes of the mevalonate pathway: hydroxymethyl glutaryl CoA (HMG-CoA) reductase, isopentenyl diphosphate (IPP) isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase, respectively. The present invention also includes at least 30 contiguous amino acids of each identified sequence or a sufficient  
15 number of contiguous amino acids to define a biologically active molecule.

The present invention also includes fragments of a polypeptide selected from SEQ ID NOs: 43, 45, 47, 49, 51, and 53. The fragment should be at least about 30 amino acids in length but must have the activity of the identified polypeptide, *e.g.*, in the case of SEQ ID NO:43, a fragment thereof that falls within the scope of the present invention has the activity of  
20 HMG-CoA reductase. As used herein, a measure of activity of the respective fragments is set forth in Example 1. A fragment having an activity above background in the assays set forth in Example 1 is considered to be biologically active and within the scope of the present invention.

The present invention also includes an amino acid sequence of a polypeptide encoded by a  
25 polynucleotide that hybridizes under stringent conditions, as defined above, to a hybridization probe that contains at least 30 contiguous nucleotides of SEQ ID NO:42 (*i.e.*, the mevalonate operon) or a complement of SEQ ID NO:42. The polynucleotide must encode at least one of the enzymes in the mevalonate pathway. For purposes of the present invention, a "hybridization probe" is a polynucleotide sequence containing from about 10-  
30 9066 nucleotides of SEQ ID NO:42.

In this embodiment, the isolated polypeptide may have the amino acid sequence of SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51 or SEQ ID NO:53. Alternatively, the isolated polypeptide may contain about 30 contiguous amino acids selected from an area of the respective amino acids sequences that have the least



identity when compared to an enzyme with the same function from different species.

Thus, for example, a polypeptide of the present invention may include amino acids 68-97 of SEQ ID NO:43, 1-30 of SEQ ID NO:45, 269-298 of SEQ ID NO:47, 109-138 of SEQ ID NO:49, 198-227 of SEQ ID NO:51 or 81-110 of SEQ ID NO:53.

- 5 Another embodiment of the invention is an isolated polypeptide having an amino acid sequence selected from: (a) an amino acid sequence shown as residues 1 to 287 of SEQ ID NO:159; (b) at least 30 contiguous amino acid residues of SEQ ID NO:159; (c) an amino acid sequence of a fragment of SEQ ID NO:159, the fragment having the activity of farnesyl-diphosphate synthase (FPP synthase); (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe containing at least 30 consecutive nucleotides of the *ispA* gene (i.e., nucleotides 295-1158 of SEQ ID NO:157) or a complement thereof, wherein the polypeptide has the activity of FPP synthase; and (e) conservatively modified variants of SEQ ID NO:159.

- Thus, in this embodiment the amino acid may be encoded by the entire open reading frame that encodes FPP synthase, i.e, residues 1-287 of SEQ ID NO:159, at least 30 contiguous residues thereof, or a fragment of SEQ ID NO:159 that has FPP synthase activity as measured by the assay set forth in Example 1. Furthermore, this embodiment of the invention also includes amino acid sequence(s) encoded by polynucleotide(s) that hybridize under stringent conditions, as defined above, to a hybridization probe that includes at least 30 consecutive nucleotides of the *ispA* gene (i.e., nucleotides 295-1158 of SEQ ID NO:157) or a complement thereof, wherein the polypeptide has FPP synthase activity as defined above.

In a preferred embodiment, the polypeptide has the amino acid sequence of SEQ ID NO:159.

- 25 Another embodiment of the invention is an isolated polypeptide having an amino acid sequence selected from the following group: (a) an amino acid sequence shown as residues 1 to 142 of SEQ ID NO:160; (b) at least 30 contiguous amino acid residues of SEQ ID NO:160; (c) an amino acid sequence of a fragment of SEQ ID NO: 160, the fragment having the activity of 1-deoxyxylulose-5-phosphate synthase (DXPS); (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe containing at least 30 consecutive nucleotides spanning positions 1185-1610 of SEQ ID NO:157 or a complement thereof, wherein the polypeptide has the activity of DXPS; and (e) conservatively modified variants of SEQ ID NO:160.

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Thus, in this embodiment the amino acid may be encoded by the entire open reading frame that encodes DXPS, i.e., residues 1-142 of SEQ ID NO:160, at least 30 contiguous residues thereof, or a fragment of SEQ ID NO:160 that has DXPS activity as measured by as measured by the assay set forth in Example 1. Furthermore, this embodiment of the invention also includes amino acid sequence(s) encoded by polynucleotide(s) that hybridize under stringent conditions, as defined above, to a hybridization probe that includes at least 30 consecutive nucleotides of the DXPS gene (i.e., nucleotides 1185-1610 of SEQ ID NO:157) or a complement thereof, wherein the polypeptide has DXPS activity as defined above.

- 10 In a preferred embodiment, the polypeptide has the amino acid sequence of SEQ ID NO:160.

Another embodiment of the invention is an isolated polypeptide having an amino acid sequence selected from: (a) an amino acid sequence shown as residues 1 to 390 of SEQ ID NO:178; (b) at least 30 contiguous amino acid residues of SEQ ID NO:178; (c) an amino acid sequence of a fragment of SEQ ID NO:178, the fragment having the activity of acetyl-CoA acetyltransferase; (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe containing at least 30 consecutive nucleotides of the *phaA* gene (i.e., nucleotides 1-1179 of SEQ ID NO:177) or a complement thereof, wherein the polypeptide has the activity of acetyl-CoA acetyltransferase, and (e) conservatively modified variants of SEQ ID NO:178.

Thus, in this embodiment the amino acid may be encoded by the entire open reading frame that encodes acetyl-CoA acetyltransferase, i.e., residues 1-143 of SEQ ID NO:178, at least 30 contiguous residues thereof, or a fragment of SEQ ID NO:178 that has acetyl-CoA acetyltransferase activity as measured by the assay set forth in Example 1. Furthermore, this embodiment of the invention also includes amino acid sequence(s) encoded by polynucleotide(s) that hybridize under stringent conditions, as defined above, to a hybridization probe that includes at least 30 consecutive nucleotides of the *phaA* gene (i.e., nucleotides 1-1170 of SEQ ID NO:177), or a complement thereof, wherein the polypeptide has the acetyl-CoA acetyltransferase activity as defined above.

- 30 In a preferred embodiment, the polypeptide has the amino acid sequence of SEQ ID NO:178.

Another embodiment of the invention is an isolated polypeptide having an amino acid sequence selected from: (a) an amino acid sequence shown as residues 1 to 240 of SEQ ID

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- NO:179; (b) at least 30 contiguous amino acid residues of SEQ ID NO:179; (c) an amino acid sequence of a fragment of a polypeptide of SEQ ID NO:179, the fragment having the activity of acetoacetyl-CoA reductase; (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe containing at least 30 consecutive nucleotides of the *phaB* gene (i.e., nucleotides 1258-1980 of SEQ ID NO:177) or a complement thereof, wherein the polypeptide has the activity of acetoacetyl-CoA reductase; and (e) conservatively modified variants of SEQ ID NO:179.

- Thus, in this embodiment the amino acid may be encoded by the entire open reading frame that encodes acetoacetyl-CoA reductase, i.e., residues 1-240 of SEQ ID NO:179, at least 30 contiguous residues thereof, or a fragment of SEQ ID NO:179 that has acetoacetyl-CoA reductase activity as measured by the assay set forth in Example 1. Furthermore, this embodiment of the invention also includes amino acid sequence(s) encoded by polynucleotide(s) that hybridize under stringent conditions, as defined above, to a hybridization probe that includes at least 30 consecutive nucleotides of the *phaB* gene (i.e., nucleotides 1258-1980 of SEQ ID NO:177) or a complement thereof, wherein the polypeptide has acetoacetyl-CoA reductase activity as defined above.

In a preferred embodiment, the polypeptide has the amino acid sequence of SEQ ID NO:179.

- The terms "polypeptide," "polypeptide sequence," "amino acid," and "amino acid sequence" are used interchangeably herein, and mean an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, as well as naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of any of the polypeptides defined herein which are at least about 30 amino acids in length and which retain some biological activity or immunological activity of the polypeptide in question. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.
- With respect to polypeptides, the term "isolated" means a protein or a polypeptide that has been separated from components that accompany it in its natural state. A monomeric protein is isolated when at least about 60 to 75% of a sample exhibits a single polypeptide sequence. An isolated protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Protein

purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel. For certain purposes, using HPLC or other means well known in the art may provide higher resolution for purification.

- 5 As used herein, the term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic polypeptide, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.
- 10 Another embodiment of the invention is an isolated polynucleotide sequence having the nucleotide sequence of the mevalonate operon (SEQ ID NO:42), variants of SEQ ID NO:42 containing one or more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (see Table 14) or fragments of SEQ ID NO:42. The variants and fragments of SEQ ID NO:42 must encode a polypeptide having an activity selected from:
  - 15 HMG-CoA reductase, isopentenyl diphosphate isomerase activity, hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase), mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions, as defined above, to a hybridization probe, the nucleotide sequence of which consists of from about 10 to about 9066 nucleotides of SEQ ID NO:42, preferably at least 30 contiguous nucleotides of SEQ ID NO:42, or
  - 20 a complement of such sequences, which polynucleotide encodes a polypeptide having an activity selected from: HMG-CoA reductase, isopentenyl diphosphate isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase.
- 25 This embodiment also includes isolated polynucleotide sequences spanning the following residues of SEQ ID NO:42: 2622 to 3644, 3641 to 4690, 4687 to 5853, 5834 to 6970, 6970 to 7887, 7880 to 8878. Fragments of these sequences are also within the scope of the invention, so long as they encode a polypeptide having HMG-CoA reductase activity, isopentenyl diphosphate isomerase activity, HMG-CoA synthase activity, mevalonate
- 30 kinase activity, phosphomevalonate kinase activity, and diphosphomevalonate decarboxylase activity, respectively.

This embodiment also includes polynucleotide sequences that hybridize under stringent conditions, as defined above, to a hybridization probe selected from a nucleotide sequence which consists of at least 30 contiguous nucleotides of the following residues of SEQ ID

NO:42: 2622 to 3644, 3641 to 4690, 4687 to 5853, 5834 to 6970, 6970 to 7887, 7880 to 8878 or a complement thereof, wherein the polynucleotide encodes a polypeptide having HMG-CoA reductase activity, isopentenyl diphosphate isomerase activity, HMG-CoA synthase activity, mevalonate kinase activity, phosphomevalonate kinase activity, or diphospho-  
5 mevalonate decarboxylase activity, respectively.

Preferably, the isolated polynucleotide consists of nucleotides 2622 to 3644, 3641 to 4690, 4687 to 5853, 5834 to 6970, 6970 to 7887 or 7880 to 8878 of SEQ ID NO:42.

Another embodiment of the invention is an isolated polynucleotide sequence having the nucleotide sequence of SEQ ID NO:157, variants of SEQ ID NO:157 containing one or  
10 more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (see Table 14) or fragments of SEQ ID NO:157 that encode a polypeptide having FPP synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity or the activity of XseB. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions, as defined above, to a hybridization probe the nucleotide sequence of which consists  
15 of at least 30 contiguous nucleotides of SEQ ID NO:157, or the complement of SEQ ID NO:157, wherein the polynucleotide encodes a polypeptide having FPP synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity or the activity of XseB.

Preferably, the isolated polynucleotide consists of nucleotides 59-292, 295-1158 or 1185-1610 of SEQ ID NO:157.

20 An isolated polynucleotide sequence is also provided that has a nucleotide sequence selected from the following group: nucleotides spanning positions 59-292 of SEQ ID NO:157, variants of the nucleotide sequence spanning positions of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of the nucleotide sequence spanning positions 59-292 of  
25 SEQ ID NO:157 that encode a polypeptide having a function of XseB, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 59-292 of SEQ ID NO:157, or the complement of such a sequence, wherein the polynucleotide encodes a polypeptide having a function of XseB.

30 Preferably, the isolated polynucleotide consists of nucleotides 59 to 292 of SEQ ID NO:157.

An isolated polynucleotide sequence is also provided that has a nucleotide sequence selected from the following group: nucleotides spanning positions 295-1158 of SEQ ID

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NO:157, variants of the nucleotide sequence spanning positions 295-1158 of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of the nucleotide sequence spanning positions 295-1158 of SEQ ID NO:157 that encode a FPP synthase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 295-1158 of SEQ ID NO:157, or the complement of such a sequence, wherein the polynucleotide encodes a polypeptide having FPP synthase activity.

Preferably, the isolated nucleotide sequence consists of nucleotides 295-1158 of SEQ ID NO:157.

Another embodiment of the invention is an isolated polynucleotide sequence having the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157, variants of the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (see Table 14) or fragments of the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157 that encode a polypeptide having 1-deoxyxylulose-5-phosphate synthase activity. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions, as defined above, to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 1185-1610 of SEQ ID NO:157, or a complement thereof, wherein the polynucleotide encodes a polypeptide having 1-deoxyxylulose-5-phosphate synthase activity.

Preferably, the isolated polynucleotide consists of nucleotides 1185 to 1610 of SEQ ID NO:157.

Another embodiment of the invention is an isolated polynucleotide sequence having the nucleotide sequence of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (see Table 14) or fragments of SEQ ID NO:177 that encode a polypeptide having an activity selected from acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions, as defined above, to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:177, or a complement thereof, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase.

- In this embodiment the isolated polynucleotide sequence may include nucleotides 1 to 1170 of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (see Table 14) or fragments of SEQ ID NO:177 that encode a polypeptide having acetyl-CoA acetyltransferase activity.
- 5 This embodiment also includes polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of nucleotides 1 to 1170 of SEQ ID NO:177, or a complement thereof, wherein the polynucleotide encodes a polypeptide having acetyl-CoA acetyltransferase activity.
- 10 Preferably, the isolated polynucleotide sequence consists of nucleotides 1-1170 of SEQ ID NO:177.

- In this embodiment, the isolated polynucleotide sequence may alternatively be nucleotides 1258-1980 of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (see Table 14) or
- 15 fragments of SEQ ID NO:177 that encode a polypeptide having acetoacetyl-CoA reductase activity. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of nucleotides 1258-1980 of SEQ ID NO:177, or a complement thereof, wherein the polynucleotide encodes a polypeptide having acetoacetyl-CoA reductase activity.
- 20

Preferably, the isolated polynucleotide consists of nucleotides 1258-1980 of SEQ ID NO:177.

- In another embodiment of the invention, the isolated polynucleotide sequence has a nucleotide sequence selected from SEQ ID NO:42, SEQ ID NO:157, SEQ ID NO:177, and
- 25 combinations thereof. As used herein, the phrase "and combinations thereof" when used in reference to nucleotide sequences means that any combination of the recited sequences may be combined to form the isolated polynucleotide sequence. Moreover, in the present invention, multiple copies of the same sequence, i.e., concatamers may be used. Likewise, and as set forth in more detail below, multiple copies of plasmids containing the same
- 30 polynucleotide sequence may be transferred into suitable host cells.

As used herein, an "isolated" polynucleotide (e.g., an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components which naturally accompany a native sequence or polypeptide, e.g., ribosomes, polymerases, many other

genome sequences and proteins. The term embraces a polynucleotide that has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems.

- 5 The phrase "nucleic acid sequence" refers to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It includes chromosomal DNA, self-replicating plasmids, infectious polymers of DNA or RNA and DNA or RNA that performs a primarily structural role.

- An "expression control sequence" is defined as an array of nucleic acid control sequences that direct transcription of an operably linked nucleic acid. An example of such an expression control sequence is a "promoter." Promoters include necessary nucleic acid sequences near the start site of transcription. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

- A polynucleotide sequence is "heterologous to" an organism or a second polynucleotide sequence if it originates from a foreign species, or, if from the same species, is modified from its original form. For example, a promoter operably linked to a heterologous coding sequence refers to a coding sequence from a species different from that from which the promoter was derived, or, if from the same species, a coding sequence which is different from any naturally occurring allelic variants.

- In the case of both expression of transgenes and inhibition of endogenous genes (e.g., by antisense, or sense suppression) one of skill will recognize that the inserted polynucleotide sequence need not be identical, but may be only "substantially identical" to a sequence of the gene from which it was derived.

In the case where the inserted polynucleotide sequence is transcribed and translated to produce a functional polypeptide, one of skill will recognize that because of codon degeneracy a number of polynucleotide sequences will encode the same polypeptide. These vari-



ants are specifically within the scope of the present invention. In addition, the present invention specifically includes those sequences that are substantially identical (determined as described below) to each other and that encode polypeptides that are either mutants of wild type polypeptides or retain the function of the polypeptide (e.g., resulting from conservative substitutions of amino acids in the polypeptide). In addition, variants can be those that encode dominant negative mutants as described below.

Two nucleic acid sequences or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described below. The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. When percentage of sequence identity is used in reference to proteins or peptides, it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions, where amino acids residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated according to, e.g., the algorithm of Meyers & Miller, Computer Applic. Biol. Sci. 4:11-17 (1988), e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, Calif., USA).

The phrase "substantially identical," in the context of two nucleic acids or polypeptides, refers to sequences or subsequences that have at least 60%, preferably 80%, most preferably 90-95%, nucleotide or amino acid residue identity when aligned for maximum correspondence over a comparison window as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. This

definition also refers to a sequence of which the complement of that sequence hybridizes to the test sequence.

- For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and
- 5 reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.
- 10 A "comparison window," as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be
- 15 conducted, e.g., by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software
- 20 Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection.
- One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show
- 25 relationship and percent sequence identity. It also plots a tree or dendrogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle, *J. Mol. Evol.* 35:351-360 (1987). The method used is similar to the method described by Higgins and Sharp, *CABIOS* 5:151-153 (1989). The program can align up to 300 sequences, each of a maximum length of 5,000
- 30 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and
- 35

their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. For example, a reference sequence can be compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps.

- Another example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm [Altschul et al., J. Mol. Biol. 215:403-410 (1990)]. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length *W* in the query sequence, which either match or satisfy some positive-valued threshold score *T* when aligned with a word of the same length in a database sequence. *T* is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity *X* from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters *W*, *T*, and *X* determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (*W*) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (*B*) of 50, expectation (*E*) of 10, *M*=5, *N*=-4, and a comparison of both strands.
- The BLAST algorithm also performs a statistical analysis of the similarity between two sequences [see, e.g., Karlin and Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)]. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (*P*(*N*)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences,

or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acid codons encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an  
5 alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid  
10 (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, or substitutions to a peptide, polypeptide, or pro-  
15 tein sequence which alters a single amino acid or a small percentage of amino acids (*i.e.* less than 20%, such as 15%, 10%, 5%, 4%, 3%, 2% or 1%) in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

20 The following six groups each contain amino acids that are conservative substitutions for one another:

- Alanine (A), Serine (S), Threonine (T);
- Aspartic acid (D), Glutamic acid (E);
- Asparagine (N), Glutamine (Q);
- 25 Arginine (R), Lysine (K);
- Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
- Phenylalanine (F), Tyrosine (Y), Tryptophan (W). (see, *e.g.*, Creighton, Proteins (1984)).

An indication that two nucleic acid sequences or polypeptides are substantially identical is  
30 that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two

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molecules or their complements hybridize to each other under stringent conditions, as described below.

The phrase "specifically hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization  
5 conditions when that sequence is present in a complex mixture (e.g., total cellular or library DNA or RNA).

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target sequence, typically in a complex mixture of nucleic acid sequences, but to no other sequences. Stringent conditions are sequence-dependent and will be  
10 different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of Principles of Hybridization and the Strategy of Nucleic Acid Assays" (1993).

Generally, highly stringent conditions are selected to be about 5-10°C lower than the  
15 thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. Low stringency conditions are generally selected to be about 15-30°C below the  $T_m$ . The  $T_m$  is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at  $T_m$ , 50% of the probes are  
20 occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0M sodium ion, typically about 0.01 to 1.0M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition  
25 of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization.

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides that they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon  
30 degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions.

In the present invention, genomic DNA or cDNA containing nucleic acids of the invention can be identified in standard Southern blots under stringent conditions using the nucleic

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acid sequences disclosed here. For the purposes of this disclosure, suitable stringent conditions for such hybridizations are those which include hybridization in a buffer of 40% formamide, 1M NaCl, 1% sodium dodecyl sulfate (SDS) at 37°C, and at least one wash in 0.2X SSC at a temperature of at least about 50°C, usually about 55°C to about 60°C, for 20 minutes, or equivalent conditions. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

A further indication that two polynucleotides are substantially identical is if the reference sequence, amplified by a pair of oligonucleotide primers, can then be used as a probe under stringent hybridization conditions to isolate the test sequence from a cDNA or genomic library, or to identify the test sequence in, e.g., a northern or Southern blot.

The present invention also includes expression vectors as defined above. The expression vectors include one or more copies of each of the polynucleotide sequences set forth above. The expression vectors of the present invention may contain any of the polynucleotide sequences defined herein, such as for example SEQ ID NO:42, or the following residues of SEQ ID NO:42: 2622 to 3644, 3641 to 4690, 4687 to 5853, 5834 to 6970, 6970 to 7887, 7880 to 8878, as well as residues 59-292, 295-1158 or 1185-1610 of SEQ ID NO:157 and residues 1-1170 or 1258-1980 of SEQ ID NO:177. The expression vectors may contain combinations of the polynucleotide sequences identified herein, such as for example, SEQ ID NO:42, SEQ ID NO:157, and SEQ ID NO:177.

The polynucleotide sequences in the expression vectors may optionally be operably linked to an expression control sequence as defined above and exemplified in the Examples.

The present invention also includes for example, the following expression vectors: pBBR-K-mev-op16-1, pBBR-K-mev-op16-2, pDS-*mvaA*, pDS-*idi*, pDS-*hcs*, pDS-*mvk*, pDS-*pmk*, pDS-*mvd*, pDS-His-*mvaA*, pDS-His-*idi*, pDS-His-*hcs*, pDS-His-*mvk*, pDS-His-*pmk*, pDS-His-*mvd*, pBBR-K-*Zea4*, pBBR-K-*Zea4-up*, pBBR-K-*Zea4-down*, pBBR-K-*PcrtE-crtE-3*, pBBR-tK-*PcrtE-mvaA*, pBBR-tK-*PcrtE-idi*, pBBR-tK-*PcrtE-hcs*, pBBR-tK-*PcrtE-mvk*, pBBR-tK-*PcrtE-pmk*, pBBR-tK-*PcrtE-mvd*, pBBR-K-*PcrtE-mvaA-crtE-3*, pDS-His-*phaA*, pBBR-K-*PcrtE-crtW*, pBBR-K-*PcrtE-crtWZ*, pBBR-K-*PcrtE-crtZW*, and combinations thereof. These expression vectors are defined in more detail in the examples below. Moreover, the present invention also includes any expression vector that contains one of the sequences defined herein, which expression vector is used to express an isoprenoid compound, such as a carotenoid, preferably zeaxanthin, in a suitable host cell.

As used herein, the phrase "expression vector" is a replicatable vehicle that carries, and is capable of mediating the expression of, a DNA sequence encoding the polynucleotide sequences set forth herein.

- In the present context, the term "replicatable" means that the vector is able to replicate in a given type of host cell into which it has been introduced. Immediately upstream of the polynucleotide sequence(s) of interest, there may be provided a sequence coding for a signal peptide, the presence of which ensures secretion of the encoded polypeptide expressed by host cells harboring the vector. The signal sequence may be the one naturally associated with the selected polynucleotide sequence or of another origin.
- 10 The vector may be any vector that may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication; examples of such a vector are a plasmid, phage, cosmid or mini-chromosome. Alternatively, the vector may be one which, when introduced in a host cell, is integrated in the host cell genome and is replicated together with the chromosome(s) into which it has been integrated. Examples of suitable vectors are shown in the examples. The expression vector of the invention may carry any of the DNA sequences of the invention as defined below and be used for the expression of any of the polypeptides of the invention defined below.
- 20

- The present invention also includes cultured cells containing one or more of the polynucleotide sequences and/or one or more of the expression vectors disclosed herein. As used herein, a "cultured cell" includes any cell capable of growing under defined conditions and expressing one or more of polypeptides encoded by a polynucleotide of the present invention. Preferably, the cultured cell is a yeast, fungus, bacterium, or alga. More preferably, the cultured cell is a *Paracoccus*, *Flavobacterium*, *Agrobacterium*, *Alcaligenes*, *Erwinia*, *E. coli* or *B. subtilis*. Even more preferably, the cell is a *Paracoccus*, such as for example, R-1506, R-1512, R1534 or R114. The present invention also includes the progeny of any of the cells identified herein that express a polypeptide disclosed herein. In the present invention, a cell is a progeny of another cell if its AFLP DNA fingerprint is indistinguishable using the conditions set forth in Example 2 from the fingerprint of the putative parental cell.
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Thus, the cultured cells according to the present invention may contain, for example, SEQ ID NO:42, or the following residues of SEQ ID NO:42: 2622 to 3644, 3641 to 4690, 4687

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to 5853, 5834 to 6970, 6970 to 7887, 7880 to 8878, as well as residues 59-292, 295-1158 or 1185-1610 of SEQ ID NO:157 and residues 1-1170 or 1258-1980 of SEQ ID NO:177.

These sequences may be transferred to the cells alone or as part of an expression vector.

These sequences also may optionally be operatively linked to expression control

- 5 sequence(s). The cultured cells may also contain combinations of the polynucleotide sequences identified herein, such as for example, SEQ ID NO:42, SEQ ID NO:157, and SEQ ID NO:177.

- The cultured cells according to the present invention may further contain polynucleotides that encode one or more enzymes in the carotenoid biosynthetic pathway. For example, 10 the cultured cells according to the present invention may contain one or more copies of SEQ ID NOs:180, 182, and 184 alone or in combination with any of the polynucleotide sequences identified herein. Thus, the polynucleotide sequences disclosed herein may be transferred into a cultured cell alone or in combination with another polynucleotide sequence that would provide enhanced production of the target isoprenoid compound, 15 such as, for example, carotenoids like zeaxanthin or astaxanthin. In this regard, the present invention includes the use of any polynucleotide encoding, for example, a polypeptide involved in carotenoid biosynthesis, such as GGPP synthase,  $\beta$ -carotene- $\beta$ 4-oxygenase (ketolase), and/or  $\beta$ -carotene hydroxylase. In addition, combinations of polynucleotides encoding polypeptides involved in carotenoid biosynthesis may be used in combination 20 with one or more of the polynucleotides identified herein on the same or different expression vectors. Such constructs may be transferred to a cultured cell according to the present invention to provide a cell that expresses an isoprenoid of interest.

- For example, a cultured cell according to the present invention may contain one or more of the following expression vectors: pBBR-K-mev-op16-1, pBBR-K-mev-op16-2, pDS- 25 *mvA*, pDS-*idi*, pDS-*hcs*, pDS-*mvk*, pDS-*pmk*, pDS-*mvd*, pDS-His-*mvA*, pDS-His-*idi*, pDS-His-*hcs*, pDS-His-*mvk*, pDS-His-*pmk*, pDS-His-*mvd*, pBBR-K-Zea4, pBBR-K-Zea4-up, pBBR-K-Zea4-down, pBBR-K-*PcrtE-crtE-3*, pBBR-tK-*PcrtE-mvA*, pBBR-tK-*PcrtE-idi*, pBBR-tK-*PcrtE-hcs*, pBBR-tK-*PcrtE-mvk*, pBBR-tK-*PcrtE-pmk*, pBBR-tK-*PcrtE-mvd*, pBBR-K-*PcrtE-mvA-crtE-3*, pDS-His-*phaA*, pBBR-K-*PcrtE-crtW*, pBBR-K-*PcrtE-crtWZ*, 30 pBBR-K-*PcrtE-crtZW*, and combinations thereof.

Another embodiment of the invention is a method of producing a carotenoid. In this method, a cultured cell as defined above is cultured under conditions permitting expression of a polypeptide encoded by the polynucleotide sequence as defined above. Culture conditions that permit expression of a polypeptide are provided in the Examples



below, but may be modified, if required, to suit the particular intended use. The carotenoid is then isolated from the cell or, if secreted, from the medium of the cell.

In the present invention, a "carotenoid" includes the following compounds: phytoene, lycopene,  $\beta$ -carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone, adonirubin, and combinations thereof. Preferably, the carotenoid is zeaxanthin.

Another embodiment of the invention is a method of making a carotenoid-producing cell. This method includes (a) introducing into a cell a polynucleotide sequence encoding an enzyme in the mevalonate pathway, which enzyme is expressed in the cell; and (b) selecting a cell containing the polynucleotide sequence of step (a) that produces a carotenoid at a level that is about 1.1-1,000 times the level of the carotenoid produced by the cell before introduction of the polynucleotide sequence.

As used herein, the phrase "an enzyme in the mevalonate pathway" means the enzymes involved in the mevalonate pathway for IPP biosynthesis and encoded by the *atoB* or *phaA*, *hcs*, *mvaA*, *mvk*, *pmk*, and *mvd* genes. For purposes of the present invention, an enzyme is "expressed in the cell" if it is detected using any one of the activity assays set forth in Example 1. Assays for detecting the production of a carotenoid are well known in the art. Examples 1, 11, and 12 provide typical assay procedures for identifying the presence of zeaxanthin, lycopene, and astaxanthin, respectively. In a similar manner, assays for the other carotenoids may be used to detect the presence in the cell or medium of e.g. phytoene, canthaxanthin, adonixanthin, cryptoxanthin, echinenone, and adonirubin.

Thus, this method may be used to make the following exemplary carotenoids: phytoene, lycopene,  $\beta$ -carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone, adonirubin, and combinations thereof. In this method, zeaxanthin is the preferred carotenoid.

This method includes producing cells capable of producing a carotenoid at a level that is about 1.1-1,000 times, preferably about 1.5-500 times, such as about 100 times or at least 10 times, the level of the carotenoid produced by the cell before introduction of the polynucleotide sequence.

In this method, the cell produces from about 1 mg/L to about 10 g/L of a carotenoid. It is preferred that the cell produces from about 100 mg/L to about 9 g/L, such as, for example, from about 500mg/L to about 8 g/L, or from about 1 g/L to about 5 g/L, of a carotenoid.

In this method, the cell may be selected from a yeast, fungus, bacterium, and alga. Preferably, the cell is a bacterium selected from *Paracoccus*, *Flavobacterium*, *Agrobacterium*, *Alcaligenes*, *Erwinia*, *E. coli*, and *B. subtilis*. More preferably, the bacterium is a *Paracoccus*.

In this method, the cell may be a mutant cell. As used herein, a "mutant cell" is any cell  
 5 that contains a non-native polynucleotide sequence or a polynucleotide sequence that has been altered from its native form (e.g., by rearrangement or deletion or substitution of from 1-100, preferably 20-50, more preferably less than 10 nucleotides). Such a non-native sequence may be obtained by random mutagenesis, chemical mutagenesis, UV-irradiation, and the like. Preferably, the mutation results in the increased expression of one  
 10 or more genes in the mevalonate pathway that results in an increase in the production of a carotenoid, such as zeaxanthin. Methods for generating, screening for, and identifying such mutant cells are well known in the art and are exemplified in the Examples below. Examples of such mutants are R114 or R1534. Preferably, the mutant cell is R114.

In this method, the polynucleotide sequence is SEQ ID NO:42, or the following residues of  
 15 SEQ ID NO:42: 2622 to 3644, 3641 to 4690, 4687 to 5853, 5834 to 6970, 6970 to 7887, 7880 to 8878, as well as residues 59-292, 295-1158 or 1185-1610 of SEQ ID NO:157 and residues 1-1170 or 1258-1980 of SEQ ID NO:177. These sequences may be used in this method alone or as part of an expression vector. These sequences also may optionally be operatively linked to expression control sequence(s). In this method, combinations of the  
 20 polynucleotide sequences identified herein may be used, such as for example, SEQ ID NO:42, SEQ ID NO:157, and SEQ ID NO:177.

Examples of expression vector that may be selected for use in this method include pBBR-K-mev-op16-1, pBBR-K-mev-op16-2, pDS-mvaA, pDS-idi, pDS-hcs, pDS-mvk, pDS-pmk,  
 pDS-mvd, pDS-His-mvaA, pDS-His-idi, pDS-His-hcs, pDS-His-mvk, pDS-His-pmk,  
 25 pDS-His-mvd, pBBR-K-Zea4, pBBR-K-Zea4-up, pBBR-K-Zea4-down, pBBR-K-PcrtE-crtE-3, pBBR-tK-PcrtE-mvaA, pBBR-tK-PcrtE-idi, pBBR-tK-PcrtE-hcs, pBBR-tK-PcrtE-mvk, pBBR-tK-PcrtE-pmk, pBBR-tK-PcrtE-mvd, pBBR-K-PcrtE-mvaA-crtE-3, pDS-His-phaA, pBBR-K-PcrtE-crtW, pBBR-K-PcrtE-crtWZ, pBBR-K-PcrtE-crtZW, and combinations thereof.

30 In this method, the polynucleotide sequence is introduced into the cell using any conventional means. Examples of suitable methods for introducing a polynucleotide sequence into a cell include transformation, transduction, transfection, lipofection, electroporation [see e.g., Shigekawa and Dower, *Biotechniques* 6:742-751 (1988)], conjugation [see e.g., Koehler and Thorne, *Journal of Bacteriology* 169:5771-5278 (1987)], and biolistics.

The use of conjugation to transfer a polynucleotide sequence, such as in the form of an expression vector, into recipient bacteria is generally effective, and is a well-known procedure. (e.g. US 5,985,623). Depending on the strain of bacteria, it may be more common to use transformation of competent cells with purified DNA.

- 5 Known electroporation techniques (both *in vitro* and *in vivo*) function by applying a brief high voltage pulse to electrodes positioned around the treatment region. (e.g. US 6,208,893). The electric field generated between the electrodes causes the cell membranes to temporarily become porous, whereupon molecules of the implant agent enter the cells. In known electroporation applications, this electric field comprises a single
- 10 square wave pulse on the order of 1000 V/cm of about 100  $\mu$ s duration. Such a pulse may be generated, for example, in known applications of the Electro Square Porator T820, made by the BTX Division of Genetronics, Inc.

- Biolistics is a system for delivering polynucleotides into a target cell using microprojectile bombardment techniques. An illustrative embodiment of a method for delivering poly-
- 15 nucleotides into target cells by acceleration is a Biolistics Particle Delivery System, which can be used to propel particles coated with DNA or cells through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with cultured target cells. The screen disperses the particles so that they are not delivered to the target cells in large aggregates. It is believed that a screen intervening between the projectile apparatus and the cells to be
  - 20 bombarded reduces the size of projectiles aggregate and may contribute to a higher frequency of transformation by reducing damage inflicted on the recipient cells by projectiles that are too large.

- For the bombardment, cells in suspension are preferably concentrated on filters or solid culture medium. Alternatively, other target cells may be arranged on solid culture
- 25 medium. The cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the acceleration device and the cells to be bombarded. Through the use of these well-known techniques one may obtain up to 1000 or more foci of cells transiently expressing a marker gene. The number of cells in a focus which express the exogenous gene product 48
  - 30 hours post-bombardment often range from 1 to 10 and average 1 to 3.

In bombardment transformation, one may optimize the prebombardment culturing conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment are important in this technology. Physical factors are those that involve manipulating the polynucleotide/-

- microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately after bombardment, the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment, and also the nature of the transforming
- 5 DNA, such as linearized DNA or intact supercoiled plasmids.

- Accordingly, it is contemplated that one may wish to adjust various of the bombardment parameters in small-scale studies to fully optimize the conditions. One may particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance, and helium pressure. One may also minimize the trauma reduction factors (TRFs) by modifying
- 10 conditions which influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted for optimum transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.
- 15 The methods of particle-mediated transformation is well known to those of skill in the art. E.g. US 5,015,580 (specifically incorporated herein by reference) describes the transformation of soybeans using such a technique.

- Another embodiment of the invention is a method for engineering a bacterium to produce an isoprenoid compound. Such a bacterium is made by (a) culturing a parent bacterium
- 20 in a medium under conditions permitting expression of an isoprenoid, and selecting a mutant bacterium from the culture medium that produces about 1.1-1,000 times more of an isoprenoid than the parent bacteria; (b) introducing into the mutant bacterium an expression vector containing a polynucleotide sequence represented by SEQ ID NO:42 operably linked to an expression control sequence; and (c) selecting a bacterium that con-
- 25 tains the expression vector and produces at least about 1.1 times more of an isoprenoid than the mutant in step (a).

In this embodiment, an isoprenoid compound means a compound structurally based on isopentenyl diphosphate (IPP) units of the formula:



- 30 Such compounds include the hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, triterpenes (e.g., phytosterols, phytoestrogens, phytoecdysones, estrogens, phytoestrogens),

tetraterpenes (carotenoids), and polyterpenes. Preferably, the isoprenoid is a carotenoid, such as for example, one of the carotenoids identified above, in particular zeaxanthin.

The bacterium may be any bacterium that is capable of producing an isoprenoid compound using the processes disclosed herein. Preferably, the bacterium is a *Paracoccus*,  
5 *Flavobacterium*, *Agrobacterium*, *Alcaligenes*, *Erwinia*, *E. coli*, or *B. subtilis*. Even more preferably, the bacterium is a *Paracoccus*. Preferably, the parent bacterium is R-1506 or R 1512, and the mutant bacterium is R1534 or R114, preferably R114.

The bacterium is cultured in a media and under conditions that are optimized for the production of the isoprenoid. The selection of media and culture conditions are well within  
10 the skill of the art. The assays set forth in Examples 1, 11, and 12 provide exemplary methods for measuring the presence of certain carotenoids in a culture media. By optimizing the culture conditions and measuring for the production of the target isoprenoid, the culturing and selection of a mutant that meets the specific production parameters recited herein may be met. In this way, a mutant bacterium producing from  
15 about 1.1-1,000 times more of an isoprenoid than the parent bacterium may be selected. Preferably, the mutant bacterium produces from about 1.5-500 times more of an isoprenoid than the parent bacterium, such as for example, at least about 100 times or at least about 10 times more of an isoprenoid than the parent bacterium. That bacterium is then cultured and used in subsequent steps.

20 After selecting the mutant bacterium that produces the desired level of an isoprenoid, an expression vector is introduced into the bacterium using any of the methods set forth above or described in the examples. Any of the expression vectors defined herein may be introduced into the mutant cell. Preferably, the expression vector contains SEQ ID NO:42.

Once the expression vector is introduced into the mutant bacteria, a stable transformant is  
25 selected that produces at least about 1.1 times, such as about 5 to about 20 times, more of an isoprenoid than the untransformed mutant. The selected transformant is then cultured under conditions suitable for isoprenoid production, and then the isoprenoid is isolated from the cell or the culture medium.

A further step in this method is introducing a mutation into the mutant bacterium that  
30 results in the increased production of an isoprenoid compound by the bacterium. The mutation may be selected from at least one of the following: inactivating the polyhydroxyalkanoate (PHA) pathway, increasing expression of acetyl-CoA acetyltransferase, increasing expression of FPP synthase, increasing expression of an enzyme in a carotenoid

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biosynthetic pathway, and increasing the expression of an enzyme for converting isopentenyl diphosphate (IPP) to dimethylallyl diphosphate (DMAPP).

The inactivating of the PHA pathway may be achieved by selecting for a mutant bacterium that does not express a polypeptide encoded by *phaB* (nucleotide positions 1258-1980 of SEQ ID NO:177) or by disrupting expression of the wild type *phaB* gene by homologous recombination using SEQ ID NO:177 or fragments thereof.

In this method, increasing expression of acetyl-CoA acetyltransferase may be achieved by introducing into the mutant bacterium a vector containing a polynucleotide sequence represented by SEQ ID NO:175 or nucleotide positions 1-1170 of SEQ ID NO:177 operably linked to an expression control sequence. In this method, increasing expression of FPP synthase may be achieved by introducing into the mutant bacterium a vector containing a polynucleotide sequence represented by nucleotides 295-1158 of SEQ ID NO:157 operably linked to an expression control sequence. In this method, increasing expression of a carotenoid gene may be achieved by introducing into the mutant bacterium a vector comprising a polynucleotide sequence that encodes one or more enzymes in the carotenoid biosynthetic pathway, such as for example a polynucleotide sequence selected from the group consisting of SEQ ID NOs:180, 182, and 184 operably linked to an expression control sequence.

In this method, it is preferred that the isoprenoid compound is isopentenyl diphosphate (IPP). It is also preferred that the isoprenoid compound is a carotenoid, such as for example, phytoene, lycopene,  $\beta$ -carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone, adonirubin, and combinations thereof.

Another embodiment of the invention is a microorganism of the genus *Paracoccus*, which microorganism has the following characteristics: (a) a sequence similarity to SEQ ID NO:12 of >97% using a similarity matrix obtained from a homology calculation using GeneCompar v. 2.0 software with a gap penalty of 0%; (b) a homology to R-1512, R1534, R114 or R-1506 of >70% using DNA:DNA hybridization at 81.5°C; (c) a G+C content of its genomic DNA that varies less than 1% from the G+C content of the genomic DNA of R114, R-1512, R1534, and R-1506; and (d) an average DNA fingerprint that clusters at about 58% similarity to strains R-1512, R1534, R114 and R-1506 using the AFLP procedure of Example 2, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966).

Methods for determining each of these characteristics are fully set forth in Example 2, and it is contemplated when these methods are used that microorganisms meeting the above criteria will be readily identifiable. It is preferred that a microorganism of the present invention have each characteristic set forth above (i.e., a-d). However, any combination of the characteristics a-d, which provides sufficient information to taxonomically validly describe a microorganism belonging to the same species as R114, R-1512, R1534, and R-1506, with the exception of *Paracoccus* sp. (MBIC3966) is also within the scope of the invention.

Another embodiment of the invention is a microorganism of the genus *Paracoccus*, which microorganism has the following characteristics: (a) 18:1w7c comprising at least about 75% of the total fatty acids of the cell membranes; (b) an inability to use adonitol, i-erythritol, gentiobiose,  $\beta$ -methylglucoside, D-sorbitol, xylitol and quinic acid as carbon sources for growth; and (c) an ability to use L-asparagine and L-aspartic acid as carbon sources for growth, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966).

Methods for determining each of these characteristics are also fully set forth in Example 2, and it is contemplated when these methods are used that microorganisms meeting the above criteria will be readily identifiable. It is preferred that a microorganism of the present invention have each characteristic set forth above (i.e., a-c). However, any combination of the characteristics a-c, which provides sufficient information to taxonomically validly describe a microorganism belonging to the same species as R114, R-1512, R1534, and R-1506, with the exception of *Paracoccus* sp. (MBIC3966) is also within the scope of the invention.

Another embodiment of the invention is a microorganism of the genus *Paracoccus*, which microorganism has the following characteristics: (a) an ability to grow at 40°C; (b) an ability to grow in a medium having 8% NaCl; (c) an ability to grow in a medium having a pH of 9.1; and (d) a yellow-orange colony pigmentation, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966).

Methods for determining each of these characteristics are also fully set forth in Example 2, and it is contemplated when these methods are used that microorganisms meeting the above criteria will be readily identifiable. It is preferred that a microorganism of the present invention have each characteristic set forth above (i.e., a-d). However, any combination of the characteristics a-d, which provides sufficient information to taxonomically validly describe a microorganism belonging to the same species as R114, R-1512, R1534,

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and R-1506, with the exception of *Paracoccus* sp. (MBIC3966) is also within the scope of the invention.

A microorganism of the present invention may also be identified using any combination of the 11 characteristics set forth above, which provide sufficient information to taxonomically validly describe a microorganism belonging to the same species as R114, R-1512, R1534, and R-1506, with the exception of *Paracoccus* sp. (MBIC3966).

In accordance with the foregoing the present invention provides

- (1) an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (a) an amino acid sequence shown as residues 1 to 340 of SEQ ID NO:43, in particular an amino acid sequence corresponding to positions 68-97 of SEQ ID NO:43;
  - (b) an amino acid sequence shown as residues 1 to 349 of SEQ ID NO:45, in particular an amino acid sequence corresponding to positions 1-30 of SEQ ID NO:45;
  - (c) an amino acid sequence shown as residues 1 to 388 of SEQ ID NO:47, in particular an amino acid sequence corresponding to positions 269-298 of SEQ ID NO:47;
  - (d) an amino acid sequence shown as residues 1 to 378 of SEQ ID NO:49, in particular an amino acid sequence corresponding to positions 109-138 of SEQ ID NO:49;
  - (e) an amino acid sequence shown as residues 1 to 305 of SEQ ID NO:51, in particular an amino acid sequence corresponding to positions 198-227 of SEQ ID NO:51;
  - (f) an amino acid sequence shown as residues 1 to 332 of SEQ ID NO:53, in particular an amino acid sequence corresponding to positions 81-110 of SEQ ID NO:53;
  - (g) a fragment of an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, wherein said fragment has at least 30 contiguous amino acid residues;
  - (h) an amino acid sequence of a fragment of a polypeptide selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, the fragment having the activity of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase), mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase;
  - (i) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides of SEQ ID NO:42 or a complement of SEQ ID NO:42, wherein the polypeptide has the activity of HMG-CoA reductase, isopentenyl diphosphate



isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; and

(j) a conservatively modified variant of SEQ ID NO:43, 45, 47, 49, 51 or 53.

- (2) an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
- (a) an amino acid sequence shown as residues 1 to 287 of SEQ ID NO:159;
  - (b) at least 30 contiguous amino acid residues of SEQ ID NO:159;
  - (c) an amino acid sequence of a fragment of SEQ ID NO: 159, the fragment having the activity of farnesyl diphosphate synthase (FPP synthase);
  - (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 295-1158 of SEQ ID NO:157 or a complement thereof, wherein the polypeptide has the activity of FPP synthase; and
  - (e) a conservatively modified variant of SEQ ID NO:159.
- (3) an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
- (a) an amino acid sequence shown as residues 1 to 142 of SEQ ID NO:160;
  - (b) at least 30 contiguous amino acid residues of SEQ ID NO:160;
  - (c) an amino acid sequence of a fragment of SEQ ID NO: 160, the fragment having the activity of 1-deoxyxylulose-5-phosphate synthase (DXPS);
  - (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1185-1610 of SEQ ID NO:157 or a complement thereof, wherein the polypeptide has the activity of DXPS;
  - (e) a conservatively modified variant of SEQ ID NO:160.
- (4) an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
- (a) an amino acid sequence shown as residues 1 to 390 of SEQ ID NO:178;
  - (b) at least 30 contiguous amino acid residues of SEQ ID NO:178;
  - (c) an amino acid sequence of a fragment of a polypeptide of SEQ ID NO: 178, the fragment having the activity of acetyl-CoA acetyltransferase;
  - (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1-1170 of SEQ ID NO:177 or a complement thereof, wherein the polypeptide has the activity of acetyl-CoA acetyltransferase; and
  - (e) a conservatively modified variant of SEQ ID NO:178.

- (5) an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
- (a) an amino acid sequence shown as residues 1 to 240 of SEQ ID NO:179;
  - (b) at least 30 contiguous amino acid residues of SEQ ID NO:179;
  - 5 (c) an amino acid sequence of a fragment of a polypeptide of SEQ ID NO: 179, the fragment having the activity of acetoacetyl-CoA reductase;
  - (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1258-1980 of SEQ ID NO:177 or a complement thereof, wherein the polypeptide has the activity of acetoacetyl-CoA reductase;
  - 10 and
  - (e) a conservatively modified variant of SEQ ID NO:179.
- (6) an isolated polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:42, variants of SEQ ID NO:42 containing one or
- 15 more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having an activity selected from the group consisting of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase), mevalonate kinase, phosphomevalonate kinase, and
- 20 diphosphomevalonate decarboxylase, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:42, or the complement of SEQ ID NO:42, which polynucleotide encodes a polypeptide having an activity
- 25 selected from the group consisting of HMG-CoA reductase, isopentenyl diphosphate isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase; in particular
- (a) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 2622 to 3644 of SEQ ID NO:42, fragments thereof that encode a polypeptide having HMG-CoA reductase activity, and poly-
  - 30 nucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 2622 to 3644 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a polypeptide having HMG-CoA reductase activity, more particularly a polynucleotide sequence consisting of nucleotides 2622 to 3644 of SEQ
  - 35 ID NO:42;
  - (b) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 3641 to 4690 of SEQ ID NO:42,

- variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having isopentenyl diphosphate isomerase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the
- 5 nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 3641 to 4690 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a polypeptide having isopentenyl diphosphate isomerase activity, more particularly a polynucleotide sequence consisting of nucleotides 3641 to 4690 of SEQ ID NO:42.
- 10 (c) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 4687 to 5853 of SEQ ID NO:42, variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having HMG-CoA synthase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of
- 15 which consists of at least 30 contiguous nucleotides spanning residues 4687 to 5853 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a polypeptide having HMG-CoA synthase activity, more particularly a polynucleotide sequence consisting of nucleotides 3641 to 4690 of SEQ ID NO:42;
- 20 (d) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 5834 to 6970 of SEQ ID NO:42, variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having mevalonate kinase activity, and polynucleotide sequences that
- 25 hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 5834 to 6970 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a polypeptide having mevalonate kinase activity, more particularly a polynucleotide sequence consisting of nucleotides 3641 to 4690 of SEQ ID NO:42;
- 30 (e) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 6970 to 7887 of SEQ ID NO:42, variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having phosphomevalonate kinase activity, and polynucleotide sequences that
- 35 hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 6970 to 7887 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a

- polypeptide having phosphomevalonate kinase activity, more particularly a polynucleotide sequence consisting of nucleotides 3641 to 4690 of SEQ ID NO:42; or
- (f) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 7880 to 8878 of SEQ ID NO:42, variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having diphosphomevalonate decarboxylase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 7880 to 8878 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a polypeptide having diphosphomevalonate decarboxylase activity, more particularly an isolated polynucleotide consisting of nucleotides 7880 to 8878 of SEQ ID NO:42, more particularly a polynucleotide sequence consisting of nucleotides 3641 to 4690 of SEQ ID NO:42;
- (7) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:157, variants of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:157 that encode a polypeptide having farnesyl diphosphate (FPP) synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity or a polypeptide having the activity of XseB, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:157, or the complement of SEQ ID NO:157, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of FPP synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity, and the activity of XseB, in particular
- (a) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of a nucleotide sequence spanning positions 59-292 of SEQ ID NO:157, variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of the nucleotide sequence spanning positions 59-292 of SEQ ID NO:157 that encode a polypeptide having the function of XseB, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 59-292 of SEQ ID NO:157, or the complement of such a sequence, wherein the polynucleotide encodes a polypeptide having the function of XseB, more particularly an isolated polynucleotide consisting of nucleotides 59 to 292 of SEQ ID NO:157;

- (b) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence spanning positions 295-1158 of SEQ ID NO:157, variants of the nucleotide sequence spanning positions 295-1158 of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of the nucleotide sequence spanning positions 295-1158 of SEQ ID NO:157 that encode a FPP synthase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 295-1158 of SEQ ID NO:157, or the complement of such a sequence, wherein the polynucleotide encodes a polypeptide having FPP synthase activity, more particularly an isolated polynucleotide consisting of nucleotides 295 to 1158 of SEQ ID NO:157;
- (c) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157, variants of the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157 that encode a polypeptide having 1-deoxyxylulose-5-phosphate synthase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 1185-1610 of SEQ ID NO:157, or the complement of such a sequence, wherein the polynucleotide encodes a polypeptide having 1-deoxyxylulose-5-phosphate synthase activity, more particularly an isolated polynucleotide consisting of nucleotides 1185 to 1610 of SEQ ID NO:157;
- (8) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:177 that encode a polypeptide having an activity selected from the group consisting of acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:177, or the complement of SEQ ID NO:177, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase, in particular

- (a) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 1 to 1170 of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:177 that encode a polypeptide having acetyl-CoA acetyltransferase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 1 to 1170 of SEQ ID NO:177, or a complement thereof, wherein the polynucleotide encodes a polypeptide having acetyl-CoA acetyltransferase activity, more particularly an isolated polynucleotide sequence consisting of nucleotides 1-1170 of SEQ ID NO:177;
- (b) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 1258-1980 of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:177 that encode a polypeptide having acetoacetyl-CoA reductase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 1258-1980 of SEQ ID NO:177, or a complement thereof, wherein the polynucleotide encodes a polypeptide having acetoacetyl-CoA reductase activity, more particularly an isolated polynucleotide sequence consisting of nucleotides 1258-1980 of SEQ ID NO:177;
- (9) an isolated polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:42, SEQ ID NO:157, SEQ ID NO:177, and combinations thereof;
- (10) an expression vector comprising the polynucleotide sequence of any one of (6) (a) to (6) (f), (7) (a) to (7) (c), (8) (a), (8) (b) or (9), in particular an expression vector wherein the polynucleotide sequence is operably linked to an expression control sequence, e.g. an expression vector further comprising a polynucleotide sequence that encodes an enzyme in the carotenoid biosynthetic pathway, more particularly an expression vector wherein the polynucleotide sequence is selected from the group consisting of SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, and combinations thereof which are operably linked to an expression control sequence;
- (11) an expression vector selected from the group consisting of pBBR-K-mev-op16-1, pBBR-K-mev-op16-2, pDS-*mvaA*, pDS-*idi*, pDS-*hcs*, pDS-*mvk*, pDS-*pmk*, pDS-*mvd*, pDS-His-*mvaA*, pDS-His-*idi*, pDS-His-*hcs*, pDS-His-*mvk*, pDS-His-*pmk*, pDS-His-*mvd*, pBBR-K-Zea4, pBBR-K-Zea4-up, pBBR-K-Zea4-down, pBBR-K-*PartE-crtE-3*,

pBBR-tK-*PcrtE-mvaA*, pBBR-tK-*PcrtE-idi*, pBBR-tK-*PcrtE-hcs*, pBBR-tK-*PcrtE-mvk*, pBBR-tK-*PcrtE-pmk*, pBBR-tK-*PcrtE-mvd*, pBBR-K-*PcrtE-mvaA-crtE-3*, pDS-*His-phaA*, pBBR-K-*PcrtE-crtW*, pBBR-K-*PcrtE-crtWZ*, pBBR-K-*PcrtE-crtZW*, and combinations thereof, in particular

- 5 (a) an expression vector selected from the group consisting of pBBR-K-mev-op16-1 and pBBR-K-mev-op16-2,
- (b) an expression vector selected from the group consisting of pBBR-K-Zea4, pBBR-K-Zea4-up, and pBBR-K-Zea4-down;
- (c) an expression vector selected from the group consisting of pBBR-K-*PcrtE-crtE-3*,
- 10 pBBR-tK-*PcrtE-mvaA*, pBBR-tK-*PcrtE-idi*, pBBR-tK-*PcrtE-hcs*, pBBR-tK-*PcrtE-mvk*, pBBR-tK-*PcrtE-pmk*, pBBR-tK-*PcrtE-mvd*, and combinations thereof;
- (d) an expression vector which is pBBR-K-*PcrtE-mvaA-crtE-3*;
- (e) an expression vector which is pDS-*His-phaA*; or
- (f) an expression vector selected from the group consisting of pBBR-K-*PcrtE-crtW*,
- 15 pBBR-K-*PcrtE-crtWZ*, and pBBR-K-*PcrtE-crtZW*;
- (12) a cultured cell comprising the polynucleotide sequence of any one of (6) (a) to (f), (7) (a) to (c), (8) (a), (8) (b) or (9), or an expression vector of (10) or (11), or a progeny of the cell, wherein the cell expresses a polypeptide encoded by the polynucleotide sequence, in particular a cell which is further characterized by a feature selected from
- 20 (a) further comprising a polynucleotide sequence that encodes an enzyme in the carotenoid biosynthetic pathway, more particularly a cultured cell wherein the polynucleotide sequence that encodes an enzyme in the carotenoid biosynthetic pathway is selected from the group consisting of SEQ ID NOs:180, 182, and 184, or a progeny of the cell, wherein the cell expresses polypeptides encoded by the polynucleotide
- 25 sequences, and
- (b) from being a member of a group selected from yeast, fungus, bacterium and alga, in particular a bacterium selected from the group consisting of *Paracoccus*, *Flavobacterium*, *Agrobacterium*, *Alcaligenes*, *Erwinia*, *E. coli*, and *B. subtilis*, more particularly *Paracoccus*, more particularly *Paracoccus* selected from the group consisting of R-1506, R-1512, R1534, and R114;
- 30 (13) a method of producing a carotenoid comprising culturing a cell of (12) under conditions permitting expression of a polypeptide encoded by the polynucleotide sequence, and isolating the carotenoid from the cell or the medium of the cell;
- (14) a method of making a carotenoid-producing cell comprising:
- 35 (a) introducing into a cell a polynucleotide sequence encoding an enzyme in the mevalonate pathway, which enzyme is expressed in the cell; and

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- (b) selecting a cell containing the polynucleotide sequence of step (a) that produces a carotenoid at a level that is about 1.1-1,000 times the level of the carotenoid produced by the cell before introduction of the polynucleotide sequence, in particular a method selected from a method characterized by a feature selected from
- 5 (i) the selecting step comprising selecting a cell containing the polynucleotide sequence of step (a) that produces a carotenoid at a level that is about 1.5-500 times, particularly about 100 times, or at least about 10 times, the level of the carotenoid produced by the cell before introduction of the polynucleotide sequence;
- 10 (ii) the cell producing from about 1 mg/L to about 10 g/L of a carotenoid.
- (iii) the cell being selected from the group consisting of a yeast, fungus, bacterium, and alga, in particular selected from the group consisting of *Paracoccus*, *Flavobacterium*, *Agrobacterium*, *Alcaligenes*, *Erwinia*, *E. coli*, and *B. subtilis*, more particularly from *Paracoccus*;
- 15 (iv) the cell in step (a) being a mutant cell, in particular being selected from the group consisting of R114 and R1534, in particular the mutant cell producing about 1.1-1,000 times, in particular about 1.5-500 times, more particularly at least about 100 times more or at least about 10 times more, the level of a carotenoid compared to its non-mutant parent;
- 20 (v) the polynucleotide sequence being selected from polynucleotide sequences of (6) (a) to (f), (7) (a) to (c), (8) (a), (8) (b) and (9), in particular wherein the polynucleotide sequence is operably linked to an expression control sequence;
- (vi) the polynucleotide sequence being an expression vector of (10) or (11);
- (vii) the introducing step being selected from the group consisting of transformation, transduction, transfection, lipofection, electroporation, conjugation, and biolistics.
- 25 (viii) the carotenoid being selected from the group consisting of phytoene, lycopene,  $\beta$ -carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone, adonirubin, and combinations thereof, in particular the carotenoid being zeaxanthin;
- 30 (15) a method for engineering a bacterium to produce an isoprenoid compound comprising:
- (a) culturing a parent bacterium in a medium under conditions permitting expression of an isoprenoid compound, and selecting a mutant bacterium from the culture medium that produces about 1.1-1,000 times more of an isoprenoid compound than
- 35 the parent bacterium;



(b) introducing into the mutant bacterium an expression vector comprising a polynucleotide sequence represented by SEQ ID NO:42 operably linked to an expression control sequence; and

5 (c) selecting a bacterium that contains the expression vector and produces at least about 1.1 times more of an isoprenoid compound than the mutant in step (a), in particular

(i) a method further comprising introducing a mutation into the mutant bacterium, more particularly a method wherein the mutation causes an effect selected from at least one of the following: inactivating the polyhydroxyalkanoate (PHA) pathway, increasing expression of acetyl-CoA acetyltransferase, increasing expression of farnesyl diphosphate (FPP) synthase, increasing expression of an enzyme in a carotenoid pathway, increasing the expression of an enzyme for converting isopentenyl diphosphate (IPP) to dimethylallyl diphosphate (DMAPP),

10 most particularly a method wherein inactivating of the PHA pathway comprises selecting for a mutant bacterium that does not express a polypeptide encoded by *phaB* (nucleotide positions 1258-1980 of SEQ ID NO: 177) or by disrupting expression of the wild type *phaB* gene by homologous recombination using SEQ ID NO:177 or a fragment thereof, or

20 a method wherein increasing expression of acetyl-CoA acetyltransferase comprises introducing into the mutant bacterium a vector comprising a polynucleotide sequence represented by SEQ ID NO:175 or nucleotide positions 1-1170 of SEQ ID NO:177 operably linked to an expression control sequence, or

a method wherein increasing expression of FPP synthase comprises introducing into the mutant bacterium a vector comprising a polynucleotide sequence represented by 25 nucleotides 295-1158 of SEQ ID NO:157 operably linked to an expression control sequence, or

a method wherein increasing expression of an enzyme in a carotenoid pathway comprises introducing into the mutant bacterium a vector comprising a polynucleotide sequence selected from the group consisting of SEQ ID NOs:180, 182, 30 and 184 operably linked to an expression control sequence;

(b) a method wherein the isoprenoid is isopentenyl diphosphate (IPP).

35 (c) a method wherein the isoprenoid is a carotenoid, in particular a method wherein the carotenoid is selected from the group consisting of phytoene, lycopene,  $\beta$ -carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone, adonirubin, and combinations thereof;

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- (d) a method wherein the parent bacterium is a *Paracoccus*, in particular R-1512 or R-1506, or R1534 or R114, in particular wherein the mutant is R114;
- (16) a microorganism of the genus *Paracoccus*, which microorganism has the following characteristics:
- 5 (i) a sequence similarity to SEQ ID NO:12 of >97% using a similarity matrix obtained from a homology calculation using GeneCompar v. 2.0 software with a gap penalty of 0%;
- a homology to strain R-1512, R1534, R114 or R-1506 of >70% using DNA:DNA hybridization at 81.5°C;
- 10 a G+C content of its genomic DNA that varies less than 1% from the G+C content of the genomic DNA of R114, R-1512, R1534, and R-1506; and
- an average DNA fingerprint that clusters at about 58% similarity to strains R-1512, R1534, R114 and R-1506 using the AFLP procedure of Example 2, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966);
- 15 (ii) 18:1w7c comprising at least about 75% of the total fatty acids of the cell membranes;
- an inability to use adonitol, i-erythritol, gentiobiose,  $\beta$ -methylglucoside, D-sorbitol, xylitol and quinic acid as carbon sources for growth; and
- an ability to use L-asparagine and L-aspartic acid as carbon sources for growth, with
- 20 the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966); or
- (iii) an ability to grow at 40°C;
- an ability to grow in a medium having 8% NaCl;
- an ability to grow in a medium having a pH of 9.1; and
- a yellow-orange colony pigmentation, with the proviso that the microorganism is not
- 25 *Paracoccus* sp. (MBIC3966).

The following examples are provided to further illustrate certain aspects of the present invention. These examples are illustrative only and are not intended to limit the scope of the invention in any way.

#### Example 1: Analytical and Biochemical Methods

##### 30 (a) Analysis of Carotenoids

Sample preparation. A solvent mixture of 1:1 dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) was first prepared. This solvent mixture was stabilized by the addition of butylated hydroxytoluene (BHT, 0.5 g/l solvent mixture). Four milliliters of the stabilized DMSO/THF mixture was added to 0.4 ml of bacterial culture in a disposable 15-ml poly-

- propylene centrifuge tube (gives a final dilution factor of 1/11). The tubes were capped and mixed using a Vortex mixer for 10 seconds each. The samples were then put on a Brinkmann Vibramix shaker for 20 minutes. The tubes were centrifuged at room temperature for 4 minutes at 4000 rpm and aliquots of the clear yellow/orange supernatant were transferred into brown glass vials for analysis by High Performance Liquid Chromatography (HPLC).

- HPLC.** A reversed phase HPLC method was developed for the simultaneous determination of astaxanthin, zeaxanthin, canthaxanthin,  $\beta$ -carotene, and lycopene. The method was also able to separate the main *cis*-isomers of zeaxanthin. Chromatography was performed using an Agilent 1100 HPLC system equipped with a thermostatted autosampler and a diode array detector. The method parameters were as follows:

- Column: YMC Carotenoid C30 column, particle size 5  $\mu$ m  
250\* 4.6mm I.D., steel  
(YMC, Part No. CT99S052546WT)
- Guard column: Pelliguard LC-18 cartridge, 20 mm  
(SUPELCO, Part No. 59654)
- Mobile phase: Methanol (MeOH)/Methyl tert-butyl ether (TBME) gradient

	% MeOH	% TBME
Start	80	20
10 min	65	35
20 min	10	90

- Run time: 28 min; Typical column pressure: 90 bar at start; Flow rate: 1.0 ml/min.;
- Detection: UV at 450 nm; Injection volume: 10  $\mu$ l; Column temperature: 15°C
- Reagents.** Methanol and TBME were HPLC grade and were obtained from EM Science and J.T. Baker, respectively. DMSO (Omnisolve) was purchased from EM Science. THF (HPLC solvent) was from Burdick and Jackson.
- Calculations.** Quantitative analyses were performed with a two level calibration using external standards (provided by Hoffmann-La Roche, Basel, Switzerland). Calculations were based on peak areas.
- Selectivity.** The selectivity of the method were verified by injecting standard solutions of the relevant carotenoid reference compounds. The target compounds (all-*trans*-carotenoids) were completely separated and showed no interference. Some minor *cis* isomers may coelute, although these potentially interfering isomers are rare and need not be considered in routine analyses. The retention times of the compounds are listed in Table 1.

Table 1. HPLC retention times for carotenoids.

Carotenoid	Retention time (min.)	Carotenoid	Retention time (min.)
Astaxanthin	6.99	Canthaxanthin	9.95
Adonixanthin	7.50	Cryptoxanthin	13.45
15- <i>cis</i> -Zeaxanthin	7.80	$\beta$ -Carotene	17.40
13- <i>cis</i> -Zeaxanthin	8.23	Lycopene	21.75
all- <i>trans</i> -Zeaxanthin	9.11		

Linearity. 25 Milligrams of all-*trans*-zeaxanthin were dissolved in 50 ml of DMSO/THF mixture (giving a final zeaxanthin concentration 500  $\mu\text{g/ml}$ ). A dilution series was prepared (final zeaxanthin concentrations of 250, 100, 50, 10, 5, 1, and 0.1  $\mu\text{g/ml}$ ) and analyzed by the HPLC method described above. A linear range was found from 0.1  $\mu\text{g/ml}$  to 250  $\mu\text{g/ml}$ . The correlation coefficient was 0.9998.

Limit of detection. The lower limit of detection for zeaxanthin by this method was determined to be 60  $\mu\text{g/l}$ . A higher injection volume and optimization of the integration parameters made it possible to lower the detection limit to approximately 5  $\mu\text{g/l}$ .

Reproducibility. The retention time for all-*trans*-zeaxanthin was very stable (relative standard deviation (RSD), 0.2 %). The peak area reproducibility, based on ten repetitive analyses of the same culture sample, was determined to be 0.17 % RSD for all *trans*-zeaxanthin and 1.0 % for cryptoxanthin.

#### 15 (b) Preparation of crude extracts and enzyme assay methods.

Preparation of crude extracts. Crude extracts of *Paracoccus* and *E. coli* were prepared by resuspending washed cell pellets in 1 ml of extraction buffer (buffer used depended on the enzyme being assayed – compositions are specified along with each enzyme assay procedure described below). Cell suspensions were placed in a 2-ml plastic vial and disrupted by agitation with glass beads using a Mini Bead Beater 8 (Biospec Products, Bartlesville, OK, USA). Disruption was performed at 4°C using a medium agitation setting. The disrupted preparations were centrifuged at 21,000 x g for 20 minutes at 4°C to sediment the cell debris, and the supernatants were used directly for enzyme assays.

Protein determinations. Protein concentrations in crude extracts were determined by the method of Bradford [Anal. Biochem. 72:248-254 (1976)] using the Bio-Rad Protein Assay

Reagent (Bio-Rad, Hercules, CA, USA). Bovine serum albumin was used as the reference protein for construction of standard curves.

Acetyl-CoA acetyltransferase assays. Crude extracts were prepared in 150 mM EPPS (N-[2-hydroxyethyl] piperazine-N'-[3-propanesulfonic acid]) buffer, pH 8.0. Assays were performed in the thiolysis direction according to the method described by Slater et al. [J. Bacteriol. 180:1979-1987 (1998)]. This assay measures the disappearance of acetoacetyl-CoA spectrophotometrically at 304 nm. Reaction mixtures contained 150 mM EPPS buffer (pH 8.0), 50 mM MgCl<sub>2</sub>, 100  $\mu$ M CoA, 40  $\mu$ M acetoacetyl-CoA and crude extract. Reactions were carried out at 30°C and were initiated by addition of crude extract. The disappearance of acetoacetyl-CoA at 304 nm was monitored using a SpectraMAX Plus plate reader (Molecular Devices Corp., Sunnyvale, CA, USA) and a quartz microtiter plate (any standard spectrophotometer can also be used). Activity (expressed as U/mg protein) was calculated using a standard curve constructed with acetoacetyl-CoA (1 unit of activity = 1  $\mu$ mol acetoacetyl-CoA consumed/min.). The lower limit of detection of Acetyl-CoA acetyltransferase activity was 0.006 U/mg.

HMG-CoA synthase assays. HMG-CoA synthase was assayed according to the method of Honda et al. [Hepatology 27:154-159 (1998)]. In this assay, the formation of HMG-CoA from acetyl-CoA and acetoacetyl-CoA is measured directly by separating the reaction product and substrates by HPLC. Crude extracts were prepared in 50 mM Tris-HCl buffer (pH 8.0). Reaction mixtures (0.1 ml) contained 50 mM Tris-HCl buffer (pH 8.0), 0.1 mM EDTA, 20 mM MgCl<sub>2</sub>, 0.1 mM acetoacetyl-CoA, 0.8 mM acetyl-CoA and crude extract. Reactions were pre-incubated for 2 minutes at 30°C before adding the crude extract. After 5 minutes of reaction at 30°C, the reactions were stopped by adding 0.2 ml of 200 mM tetra-butyl ammonium phosphate (TBAP, dissolved in methanol-water (3:2, final pH was 5.5) and containing 0.2 mM propionyl-CoA as an internal recovery standard). The mixture was then centrifuged for 3 minutes at 21,000 x g at 4°C and subsequently kept on ice until analyzed by reversed phase ion-pair HPLC. HMG-CoA and propionyl-CoA were separated from acetyl-CoA and acetoacetyl-CoA using a Nova-Pak C18 column (3.9 x 150 mm, Waters Corporation, Milford, MA, USA). The injection volume was 20  $\mu$ l, the mobile phase was 50 mM TBAP dissolved in methanol-water (1:1, final pH was 5.5), and the flow rate was 1.0 ml/min. HMG-CoA and propionyl-CoA were detected by absorbance at 254 nm. HMG-CoA produced in the reaction was quantified by comparison with a standard curve created using authentic HMG-CoA. Activity is defined as U/mg protein. One unit of activity = 1 nmol HMG-CoA produced/min. The lower limit of detection of HMG-CoA synthase was about 1 U/mg.

- HMG-CoA reductase assays. Crude extracts were prepared in 25 mM potassium phosphate buffer (pH 7.2) containing 50 mM KCl, 1 mM EDTA and a protease inhibitor cocktail (Sigma Chemical Co., St. Louis, MO, USA, catalog #P-2714). Assays were performed according to the method of Takahashi et al. [J. Bacteriol. 181:1256-1263 (1999)]. This
- 5 assay measures the HMG-CoA dependent oxidation of NADPH spectrophotometrically at 340nm. Reaction mixtures contained 25 mM potassium phosphate buffer (pH 7.2), 50 mM KCl, 1 mM EDTA, 5 mM dithiothreitol, 0.3 mM NADPH, 0.3 mM R,S-HMG-CoA and crude extract. Reactions were performed at 30°C and were initiated by the addition of HMG-CoA. HMG-CoA-dependent oxidation of NADPH was monitored at 340 nm using
- 10 a SpectraMAX Plus plate reader (Molecular Devices Corp., Sunnyvale, CA, USA) and a quartz microtiter plate (any standard spectrophotometer may be used). Activity (expressed as U/mg protein) was calculated using a standard curve constructed with NADPH (1 unit of activity = 1  $\mu$ mol NADPH oxidized/min.). The lower limit of detection of HMG-CoA reductase activity was 0.03 U/mg.
- 15 Mevalonate kinase, phosphomevalonate kinase and mevalonate diphosphate decarboxylase assays. The preparation of substrates and the assay procedures for mevalonate kinase, phosphomevalonate kinase and mevalonate diphosphate decarboxylase have been described in detail by Popják [Methods Enzymol. 15:393-425 (1969)]. For all assays, one unit of enzyme activity is defined as 1 $\mu$ mol of product formed/minute. In addition to
- 20 these spectrophotometric and radiochromatographic assays, alternate methods, for example using HPLC separation of reaction substrates and products, can be used. The lower limit of detection of mevalonate kinase, phosphomevalonate kinase and mevalonate diphosphate decarboxylase is typically about 0.001 U/mg protein.
- IPP isomerase assays. Crude extracts were prepared in 50 mM Tris-HCl buffer (pH 7.5).
- 25 Assays were performed using the method of Spurgeon et al. [Arch. Biochem. Biophys. 230:445-454 (1984)]. This assay is based on the difference in acid-lability of IPP and DMAPP. Reaction mixtures (0.1 ml final volume) contained 50 mM Tris-HCl buffer (pH 7.5), 2 mM dithiothreitol, 5 mM MgCl<sub>2</sub>, 20  $\mu$ M [1-<sup>14</sup>C]-IPP and crude extract. Reactions were carried out at 30°C for 15 minutes and terminated by the addition of 0.3 ml of a mixture
- 30 of concentrated HCl:methanol (4:1) and an additional incubation at 37°C for 20 min. Hexane (0.9 ml) was added and the tubes were mixed (4 times for 10 seconds using a vortex mixer). After centrifugation (21,000 x g, 5 minutes), 0.6 ml of the hexane layer was transferred to a scintillation vial, scintillation fluid was added, and the radioactivity counted. Activity is expressed as U/mg protein. One unit of activity = 1 pmol [1-<sup>14</sup>C]-IPP incorporated into acid labile products/min. The lower limit of detection of IPP isomerase activity was 1 U/mg.

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FPP synthase assays. Crude extracts were prepared in 50 mM Tris-HCl buffer (pH 8.0). The FPP synthase assay procedure was similar to the IPP isomerase assay described above, being based on the difference in acid lability of IPP and FPP (Spurgeon et al., supra). Reaction mixtures (0.1 ml final volume) contained 50 mM Tris-HCl buffer (pH 8.0),

5 2 mM dithiothreitol, 5 mM  $MgCl_2$ , 20  $\mu M$  [ $1-^{14}C$ ]-IPP, 25  $\mu M$  GPP (geranyl pyrophosphate) and crude extract. Reactions were carried out at 30°C for 15 minutes and terminated by the addition of 0.3 ml of a mixture of concentrated HCl:methanol (4:1) and an additional incubation at 37°C for 20 minutes. Hexane (0.9 ml) was added and the tubes were mixed (4x, 10 seconds using a vortex mixer). After centrifugation (21,000 x g, 5

10 minutes), 0.6 ml of the hexane layer was transferred to a scintillation vial, scintillation fluid was added, and the radioactivity counted. Units of enzyme activity, and the lower limit of detection, were the same as defined above for IPP isomerase. In cases where high IPP isomerase activity interferes with measurement of FPP synthase activity, crude extract may be preincubated for 5 minutes in the presence of 5mM iodoacetamide to inhibit IPP

15 isomerase activity.

GGPP synthase assays. Crude extracts were prepared in 50 mM Tris-HCl buffer (pH 8.0) containing 2 mM dithiothreitol. GGPP synthase was assayed according to the procedure of Kuzuguchi et al. [J. Biol. Chem. 274:5888-5894 (1999)]. This assay is based on the same principle as described above for FPP synthase. Reaction mixtures (0.1 ml final volume)

20 contained 50 mM Tris-HCl buffer (pH 8.0), 2 mM dithiothreitol, 5 mM  $MgCl_2$ , 20  $\mu M$  [ $1-^{14}C$ ]-IPP, 25  $\mu M$  FPP and crude extract. All reaction conditions and subsequent treatment of samples for scintillation counting were identical to those described above for FPP synthase. Treatment of extract with iodoacetamide to inhibit IPP isomerase activity may also be used as above. Units of enzyme activity, and the lower limit of detection, were the same

25 as defined above for IPP isomerase.

Acetoacetyl-CoA reductase assays. Crude extracts are prepared in 50 mM Tris-HCl buffer (pH 7.5) containing 50 mM KCl and 5 mM dithiothreitol. Acetoacetyl-CoA reductase was assayed according to the procedure of Chohan and Copeland [Appl. Environ. Microbiol. 64:2859-2863 (1998)]. This assay measures the acetoacetyl-CoA-dependent oxidation of

30 NADPH spectrophotometrically at 340 nm. Reaction mixtures (1 ml) contain 50 mM Tris-HCl buffer (pH 8.5), 15 mM  $MgCl_2$ , 250  $\mu M$  NADPH, and 100  $\mu M$  acetoacetyl-CoA. Reactions are performed in a quartz cuvette at 30°C and are initiated by the addition of acetoacetyl-CoA. Activity (expressed as U/mg protein) was calculated using a standard curve constructed with NADPH (1 unit of activity = 1  $\mu mol$  NADPH oxidized/min). The

35 lower limit of detection of acetoacetyl-CoA reductase activity is about 0.01 U/mg.

**Example 2: Taxonomic Reclassification of *Flavobacterium* sp. as *Paracoccus***

- This Example describes the taxonomic re-classification of the zeaxanthin-producing bacterium formerly designated *Flavobacterium* sp. strain R-1512 (ATCC 21588) as *Paracoccus* sp. strain R-1512 (ATCC 21588). A comprehensive genomic and
- 5 biochemical/physiological analysis was performed by the Belgian Coordinated Collections of Microorganisms/Laboratorium voor Microbiologie, Universiteit Gent (BCCM™/LMG), using state-of-the-art methods currently accepted as the scientific standards for bacterial classification. Besides *Paracoccus* sp. strain R-1512, several other bacteria belonging to the genus *Paracoccus* were included in the study (summarized in Table 2).

10 Table 2. Bacteria used in taxonomic study.

Bacterium	Strain designation	Source or reference
<i>Paracoccus</i> sp.	R-1512 (ATCC 21588)	ATCC (environmental isolate) US 3,891,504
<i>Paracoccus</i> sp.	R1534	mutant derived from R-1512; US 6,087,152
<i>Paracoccus</i> sp.	R114	mutant derived from R-1512; This work
<i>Paracoccus</i> sp.	R-1506	environmental isolate; This work
<i>Paracoccus</i> sp.	MBIC3024	H. Kasai, Kamaishi Institute, Japan
<i>Paracoccus</i> sp.	MBIC3966	H. Kasai, Kamaishi Institute, Japan
<i>Paracoccus</i> sp.	MBIC4017	H. Kasai, Kamaishi Institute, Japan
<i>Paracoccus</i> sp.	MBIC4020	H. Kasai, Kamaishi Institute, Japan
<i>P. marcusii</i>	DSM 11574 <sup>T</sup>	Harker et al., infra
<i>P. carotinifaciens</i>	E-396 <sup>T</sup>	Tsubokura et al., infra
<i>P. solventivorans</i>	DSM 6637 <sup>T</sup>	Siller et. al., Int. J. Syst. Bacteriol. 46:1125-1130 (1996)

Strains R1534 and R114 are mutants derived from strain R-1512 by classical mutagenesis and screening for improved zeaxanthin production. The primary screening was accomplished by selecting the highest color intensity producing colonies. A secondary screening



- was accomplished in liquid culture media by the HPLC methods according to Example 1. Strain R-1506 is an independent isolate obtained from the same initial screening of environmental microorganisms that provided strain R-1512. Strains MBIC3024, MBIC3966, MBIC4017 and MBIC4020 were identified as members of the genus *Paracoccus* by the
- 5 nucleotide sequences of their 16S rDNA genes (DNA sequences were deposited in the public EMBL database, see Table 5). *Paracoccus marcusii* DSM 11574<sup>T</sup> and *Paracoccus carotinifaciens* E-396<sup>T</sup> are recently described type strains of carotenoid-producing bacteria [Harker et al., Int. J. Syst. Bacteriol. 48:543-548 (1998); Tsubokura et al., Int. J. Syst. Bacteriol. 49:277-282 (1999)]. *Paracoccus solventivorans* DSM 6637<sup>T</sup> was included as a
- 10 "control" strain, being a member of the genus *Paracoccus* but distantly related to the other bacteria used.

- Preliminary experiments resulted in the following conclusions. Each of the methods set forth herein has a well-recognized ability to define taxonomic relatedness or relative degree of similarity between organisms. The methods and their use for delineating bacterial taxa
- 15 were described and compared in detail by Van Damme et al., Microbiological Reviews 60:407-438 (1996) and Janssen et al., Microbiology 142:1881-1893 (1996).

- (1) Fatty acid analysis of the cell membranes of strains R1534 and R114 showed that the two strains were highly similar and indicated a taxonomic relatedness of these strains to *Paracoccus denitrificans* and *Rhodobacter capsulatus*.
- 20 (2) One-dimensional gel electrophoresis of cellular proteins showed a high similarity (i.e., a relatedness at the intra-species level) between R1534 and R114, but the profiles did not justify allocation of these strains to either *R. capsulatus* or *P. denitrificans*.
- (3) DNA:DNA hybridization between strain R1534 and *R. capsulatus* LMG2962<sup>T</sup> and *P. denitrificans* LMG4218<sup>T</sup> confirmed that strain R1534 is neither *R. capsulatus* nor *P. denitri-*
- 25 *ficans*.
- (4) Sequencing of 16S rDNA genes from strains R1534 and R114 showed that these organisms belong to the genus *Paracoccus*, but that they represent a new species. The highest degree of sequence similarity was observed with the 16S rDNA gene of *Paracoccus* sp. strains MBIC3966, MBIC4020 and MBIC3024.
- 30 (5) DNA fingerprinting of strains R1534 and R-1512 using Amplified Fragment Length Polymorphism (AFLP<sup>TM</sup>) showed high overall similarity of the genomic DNA from the two strains, indicating an infraspecific relatedness (i.e. AFLP<sup>TM</sup> can differentiate between two members of the same species).

In the following sections, the results and conclusions of the present comprehensive taxonomic study of *Paracoccus* sp. strain R-1512 (and its mutant derivatives R1534 and R114) are set forth.

- 16S rDNA sequencing and phylogenetic study. The bacteria set forth in Table 2 were grown in LMG medium 185 ((TSA) BBL 11768 supplemented where necessary with 1.5% Difco Bacto agar). Genomic DNA was prepared according to the protocol of Niemann et al. [J. Appl. Microbiol. 82:477-484 (1997)]. Genes coding for 16S rDNA were amplified from genomic DNA from strains R-1512, R1534, R114 and R-1506 by polymerase chain reaction (PCR) using the primers shown in Table 3.

- Table 3. Primers used for PCR amplification of DNA coding for 16S rDNA in *Paracoccus* sp. strains R-1512, R1534, R114, and R-1506.

Primer name <sup>a</sup>	Sequence (5'→3')	SEQ ID NO	Position <sup>b</sup>
16F27	AGA GTT TGA TCC TGG CTC AG	SEQ ID NO:1	8-27
16F38	CTG GCT CAG GAC/T GAA CGC TG	SEQ ID NO:2	19-38
16R1522	AAG GAG GTG ATC CAG CCG CA	SEQ ID NO:3	1541-1522

- <sup>a</sup>F, forward primer; R, reverse primer. Forward primer 16F27 (Synonym: PA) was used for strains R1534 and R-1506, while forward primer 16F38 (Synonym: ARI C/T) was used for strains R-1512 and R114. The reverse primer 16R1522 (Synonym: PH) was used for all strains.

<sup>b</sup>Hybridization position referring to *E. coli* 16S rDNA gene sequence numbering.

- The PCR-amplified DNAs were purified using the Qiaquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). Complete sequencing was performed using an Applied Biosystems, Inc. 377 DNA Sequencer and the protocols of the manufacturer (Perkin-Elmer, Applied Biosystems Division, Foster City, CA, USA) using the "ABI PRISM™ Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (with AmpliTaq® DNA Polymerase, Fs)". The primers used for DNA sequencing are shown in Table 4.

Table 4. Primers used for sequencing PCR-amplified segments of genes coding for 16S rDNA in *Paracoccus* sp. strains R-1512, R1534, R114 and R-1506.

Primer name <sup>a</sup> / Synonym	Sequence (5'→3')	SEQ ID NO	Position <sup>b</sup>
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16F358/*Gamma	CTC CTA CGG GAG GCA GCA GT	SEQ ID NO:4	339-358
16F536/*PD	CAG CAG CCG CGG TAA TAC	SEQ ID NO:5	519-536
16F926/*O	AAC TCA AAG GAA TTG ACG G	SEQ ID NO:6	908-926
16F1112/*3	AGT CCC GCA ACG AGC GCA AC	SEQ ID NO:7	1093-1112
16F1241/*R	GCT ACA CAC GTG CTA CAA TG	SEQ ID NO:8	1222-1241
16R339/Gamma	ACT GCT GCC TCC CGT AGG AG	SEQ ID NO:9	358-339
16R519/PD	GTA TTA CCG CGG CTG CTG	SEQ ID NO:10	536-519
16R1093/3	GTT GCG CTC GTT GCG GGA CT	SEQ ID NO:11	1112-1093

\*F, forward primer; R, reverse primer.

<sup>b</sup>Hybridization position referring to *E. coli* 16S rDNA gene sequence numbering.

- Five forward and three reverse primers were used to obtain a partial overlap of sequences, ensuring highly reliable assembled sequence data. Sequence assembly was performed
- 5 using the program AutoAssembler (Perkin-Elmer, Applied Biosystems Division, Foster City, CA, USA). Phylogenetic analysis was performed using the software package Gene-Compa<sup>TM</sup> (v. 2.0, Applied Maths B.V.B.A., Kortrijk, Belgium) after including the consensus sequences (from strains R-1512, R1534, R114 and R-1506) in an alignment of small ribosomal subunit sequences collected from the international nucleotide sequence library
- 10 EMBL. This alignment was pairwise calculated using an open gap penalty of 100% and a unit gap penalty of 0%. A similarity matrix was created by homology calculation with a gap penalty of 0% and after discarding unknown bases. A resulting tree was constructed using the neighbor-joining method.

- The nucleotide sequence of the 16S rDNA gene from *Paracoccus* sp. strain R-1512 is
- 15 illustrated as SEQ ID NO:12. The distance matrix, presented as the percentage of 16S rDNA sequence similarity, between strain R-1512 and its closest relatives, is shown in Table 5. The sequences from strains R-1512 and its mutant derivatives R1534 and R114 were identical. The sequence from R-1506 differed by only one nucleotide from the sequence from latter strains. This demonstrated strains R-1512 and R-1506 are phylo-
- 20 genetically highly related and likely belong to the same species (confirmed by DNA:DNA hybridization, see below). Comparison of the R-1512 and R-1506 sequences with those publicly available at the EMBL library located R-1512 and R-1506 in the genus *Paracoccus*. However, the sequence similarities observed with all currently taxonomically validly described *Paracoccus* species was <97%, the value generally accepted as the limit for a
- 25 possible relatedness at the species level [Stackebrandt and Goebel, Int. J. Syst. Bacteriol.

- 44:846-849 (1994)]. This demonstrated that strains R-1512 (and its mutant derivatives) and R-1506 belong to one or two new *Paracoccus* species. Sequence similarities of >97% (significant for a possible relationship at the species level), were observed between four unnamed *Paracoccus* strains and strains R-1512, R1534, R114 and R-1506, suggesting that one or more of the unnamed (MBIC) strains may relate at the species level to strains R-1512 and R-1506. Based on cluster analysis (phylogenetic tree depicting the phylogenetic relatedness between *Paracoccus* sp. strains R-1512, R1534, R114, R-1506, MBIC3966, and other members of the genus *Paracoccus*), strains R-1512, R1534, R114, R-1506 and four unnamed *Paracoccus* strains (MBIC3024, MBIC3966, MBIC4017 and MBIC4020) were selected for DNA:DNA hybridization experiments to analyze species relatedness.

Table 5. Distance matrix, presented as the percentage of 16S rDNA sequence similarity, between *Paracoccus* sp. strain R-1512 and its closest relatives.

Strain <sup>a</sup>	EMBL Accession number	% Similarity
R-1512	-	100
R1534	-	100
R114	-	100
R-1506	-	99.9
<i>Paracoccus</i> sp. MBIC3966	AB018688	100
<i>Paracoccus</i> sp. MBIC3024	AB008115	98.2
<i>Paracoccus</i> sp. MBIC4020	AB025191	98.1
<i>Paracoccus</i> sp. MBIC4036	AB025192	97.0
<i>Paracoccus</i> sp. MBIC4017	AB025188	96.9
<i>Paracoccus</i> sp. MBIC4019	AB025190	96.8
<i>Paracoccus</i> sp. MBIC4018	AB025189	96.4
<i>Paracoccus marcusii</i> DSM 11574 <sup>T</sup>	Y12703	96.2
<i>Paracoccus carotinifaciens</i> E-396 <sup>T</sup>	AB006899	96.1
<i>Paracoccus solventivorans</i> DSM 6637 <sup>T</sup>	Y07705	95.4
<i>Paracoccus thiocyanaticus</i> THIO11 <sup>T</sup>	D32242	95.3
<i>Paracoccus aminophilus</i> JCM 7686 <sup>T</sup>	D32239	95.1
<i>Paracoccus alcaliphilus</i> JCM 7364 <sup>T</sup>	D32238	95.0

<i>Paracoccus pantotrophicus</i> ATCC 35512 <sup>T</sup>	Y16933	95.0
<i>Paracoccus denitrificans</i> ATCC 17741 <sup>T</sup>	Y16927	94.8
<i>Paracoccus versutus</i> IAM 12814 <sup>T</sup>	D32243	94.7
<i>Paracoccus kocurii</i> JCM 7684 <sup>T</sup>	D32241	94.6
<i>Paracoccus aminovorans</i> JCM 7685 <sup>T</sup>	D32240	94.4
<i>Paracoccus alkenifer</i> A901/1 <sup>T</sup>	Y13827	94.3
<i>Rhodobacter capsulatus</i> ATCC 11166 <sup>T</sup>	D16428	92.9

<sup>a</sup>Type strains are followed by a <sup>T</sup>

- DNA:DNA hybridization and determination of G+C content. The bacteria set forth in Table 5 were grown in LMG medium 185. Genomic DNA was prepared according to the protocol of Wilson [In Ausabel et al. (eds.), Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York, 2.4.1-2.4.5 (1987)]. The G+C content of the DNA's was determined by HPLC according to Mesbach et al. [Int. J. Syst. Bacteriol. 39:159-167 (1989)] as modified by Logan et al. [Int. J. Syst. Evol. Microbiol. 50:1741-1753 (2000)]. Reported values are the mean of these measurements on the same DNA sample. DNA:DNA hybridizations were performed using the initial renaturation rate method as described by De Ley et al. [Eur. J. Biochem. 12:133-142 (1970)]. The hybridization temperature was 81.5°C. For this method, an average deviation of +/-5.8% has been reported by Vauterin et al. [Int. J. Syst. Bacteriol. 45:472-489 (1995)]. The G+C content of the bacterial DNA's and the results of the DNA hybridization experiments are summarized in Table 6.
- 15 Table 6. G+C content (mol %) of DNA from *Paracoccus* spp. strains and per cent DNA homology between the strains.

Strain	%G+C	% DNA Homology							
R-1512	67.6	100							
R1534	67.7	96	100						
R114	67.5	100	97	100					
R-1506	67.5	94	90	88	100				
MBIC3024	65.4	31	nd <sup>a</sup>	nd	31	100			
MBIC3966	66.9	93	nd	nd	88	32	100		
MBIC4017	67.2	32	nd	nd	31	24	24	100	

MBIC4020	68.4	27	nd	nd	25	25	23	34	100
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<sup>a</sup>not determined

- Strains R-1512, R1534, R114, R-1506 and MBIC3966 showed a DNA homology of >70% (the generally accepted limit for species delineation [Wayne et al., Int. J. Syst. Bacteriol. 37:463-464 (1987)]), and therefore belong to the same species within the genus *Paracoccus*.
- 5 The G+C content of these five strains varied from 66.9%-67.7%, thus remaining within 1%, characteristic for a well defined species. On the other hand, the low DNA homology between strains MBIC3024, MBIC4017 and MBIC4020 and strains R-1512, R1534, R114, R-1506 and MBIC3966 showed that MBIC3024, MBIC4017 and MBIC4020 each belong to a different genomic species within the genus *Paracoccus*.
- 10 DNA fingerprinting using AFLP™. AFLP™ is a PCR-based technique for whole genome DNA fingerprinting via the selective amplification and selective visualization of restriction fragments [Vos et al., Nucleic Acids Research 23:4407-4414 (1995); Janssen et al., supra]. In this analysis, *Paracoccus* sp. strains R-1512, R1534, R114, R-1506, MBIC3966, and *Paracoccus marcusii* DSM 11574<sup>T</sup> were compared to evaluate infraspecies relatedness. These
- 15 bacteria were grown in LMG medium 185. Genomic DNA from each of these bacteria was prepared according to the protocol of Wilson (supra). Purified DNA was digested by two restriction enzymes, a 4-base cutter and a 6-base cutter. In this way, a limited number of fragments with two different ends and of suitable size for efficient PCR were obtained. Adaptors (small double-stranded DNA molecules of 15-20 bp) containing one compatible
- 20 end were ligated to the appropriate "sticky" end of the restriction fragments. Both adaptors are restriction halfsite-specific, and have different sequences. These adaptors serve as binding sites for PCR primers. Here, the restriction enzymes used were *ApaI* (a hexacutter, recognition sequence GGGCC/C) and *TaqI* (a tetracutter, recognition sequence T/GCA). The sequences of the adaptors ligated to the sticky ends generated by
- 25 cleavage with the restriction enzymes are shown in Table 7 (SEQ ID Nos:13-22). PCR was used for selective amplification of the restriction fragments. The PCR primers specifically annealed with the adaptor ends of the restriction fragments. Because the primers contain, at their 3' end, one so-called "selective base" that extends beyond the restriction site into the fragment, only those restriction fragments that have the appropriate complementary
- 30 sequence adjacent to the restriction site were amplified. The sequences of the six PCR primer combinations used are also shown in Table 7.

Table 7. Adaptors and PCR primers used for AFLP™ analysis.

	Sequence	SEQ ID NO
Adaptors corresponding to restriction enzyme <i>Apal</i>		
Adaptor 93A03	5'-TCGTAGACTGCGTACAGGCC-3'	SEQ ID NO:13
Adaptor 93A04	3'-CATCTGACGCATGT-5'	SEQ ID NO:14
Adaptors corresponding to restriction enzyme <i>TaqI</i>		
Adaptor 94A01	5'-GACGATGAGTCCTGAC-3'	SEQ ID NO:15
Adaptor 94A02	3'-TACTCAGGACTGGC-5'	SEQ ID NO:16
	Sequence	SEQ ID NO
Primer combination 1 (PC A)		
A01	5'GACTGCGTACAGGCCCA3'	SEQ ID NO:17
T01	5'CGATGAGTCCTGACCGAA3'	SEQ ID NO:18
Primer combination 2 (PC B)		
A01	5'GACTGCGTACAGGCCCA3'	SEQ ID NO:17
T02	5'CGATGAGTCCTGACCGAC3'	SEQ ID NO:19
Primer combination 3 (PC D)		
A02	5'GACTGCGTACAGGCCCC3'	SEQ ID NO:20
T01	5'CGATGAGTCCTGACCGAA3'	SEQ ID NO:18
Primer combination 4 (PC I)		
A03	5'GACTGCGTACAGGCCCG3'	SEQ ID NO:21
T03	5'CGATGAGTCCTGACCGAG3'	SEQ ID NO:22
Primer combination 5 (PC G)		
A03	5'GACTGCGTACAGGCCCG3'	SEQ ID NO:21
T01	5'CGATGAGTCCTGACCGAA3'	SEQ ID NO:18
Primer combination 6 (PC H)		
A03	5'GACTGCGTACAGGCCCG3'	SEQ ID NO:21
T02	5'CGATGAGTCCTGACCGAC3'	SEQ ID NO:19

Following amplification, the PCR products were separated according to their length on a high resolution polyacrylamide gel using a DNA sequencer (ABI 377). Fragments that contained an adaptor specific for the restriction halfsite created by the 6-bp cutter were

visualized by autoradiography due to the 5'-end labeling of the corresponding primer with <sup>32</sup>P. The electrophoretic patterns were scanned and numerically analyzed with Gel-Compar™ 4.2 software (Applied Maths, B.V.B.A., Kortrijk, Belgium) and clustered using the Pearson curve matching coefficient and unweighted pair group averages linking [clustering methods were reviewed by Sneath and Sokal, In: Numerical Taxonomy. Freeman & Son, San Francisco (1973)].

In all six primer combinations (PC A-H, Table 7), the DNA fingerprints of *Paracoccus* sp. strains R-1512, R1534 and R114 were highly similar if not identical. In cases where minor differences were observed, reproducibility was not evaluated. The high similarity or identity among the three strains was expected as strains R1534 and R114 were derived from strain R-1512. With all primer combinations, strains R-1512, R1534 and R114 were clearly discriminated from strains R-1506 and MBIC3966, the latter two strains equally belonging to the new *Paracoccus* species. However, the fingerprints provide no clear indication that strains R-1512, R1534 and R114 are more related to either R-1506 or MBIC3966. Under the conditions used, the five strains of the new species cluster at an average level of about 58% similarity (this value is the mean of the six values of the branching points of the new species in the six AFLP™ experiments (six primer combinations)), and the cluster can clearly be discriminated from the profile of *Paracoccus marcusii* DSM 11574<sup>T</sup>, the type strain of a phylogenetically related carotenoid-producing *Paracoccus* species. The mean similarity value of the six branching points for *Paracoccus marcusii* DSM 11574<sup>T</sup> and the new species was about 11%.

Fatty acid analysis. The fatty acid composition of the cell membranes of *Paracoccus* sp. strains R-1512, R1534, R114, R-1506, MBIC3966 were compared to the type strains *P. marcusii* DSM 11574<sup>T</sup>, *P. carotinifaciens* E-396<sup>T</sup> and *P. solventivorans* DSM 6637<sup>T</sup>. The bacteria were grown for 24 hours at 28°C in LMG medium 185. The fatty acid compositions were determined by gas chromatography using the commercial system MIDI (Microbial Identification System, Inc., DE, USA). Extraction and analysis of fatty acids was performed according to the recommendations of the MIDI system. Table 8 summarizes the results for all strains tested. For the five strains of the new *Paracoccus* species (R-1512, R1534, R114, R-1506, MBIC3966), the mean profile was calculated. All eight organisms showed a comparable fatty acid composition of their cell membranes, with 18:1 w7c as the major compound. Only minor differences in fatty acid composition were observed between the new *Paracoccus* species and the three type strains.



- Utilization of carbon sources for growth For testing the aerobic utilization of carbon sources, BIOLOG-SF-N Microplate microtiter plates (Biolog Inc., Hayward, CA, USA) containing 95 substrates were used with the exception that the substrate in well E6 was D,L-lactic acid methyl ester instead of the usual sodium salt of D,L-lactic acid. Cells from
- 5 each of the strains identified in Table 9 were grown for 24 hours at 28°C in LMG medium 12 (Marine Agar, Difco 0979). A cell suspension with a density equivalent to 0.5 McFarland units was prepared in sterile distilled water. From this suspension, 18 drops were transferred into 21 ml of AUX medium (API 20NE, bioMérieux, France) and mixed gently. 0.1 Milliliters of the suspension was transferred to each well of the BIOLOG
- 10 MicroPlates, and the plates were incubated at 30°C. Wells were visually checked for growth after 48 hours and after 6 days. Also, at 6 days the visual scoring was confirmed by reading the microtiter plates using the BIOLOG plate reader.

- The results of the BIOLOG analysis are shown in Table 9. Growth (positive reaction) was determined as increased turbidity compared to the reference well without substrate. A
- 15 distinction was made between good growth (+), weak growth (±) and no growth (-). Results in parentheses are those obtained after 6 days if different from the results obtained after 48 hours. A question mark indicates an unclear result at 6 days. Of the 95 carbon sources tested, 12 could be used, and 47 could not be used, for growth by all five strains comprising the new *Paracoccus* species (R-1512, R1534, R114, R-1506 and MBIC3966).
- 20 These five strains gave variable growth responses to the remaining 36 substrates. The new *Paracoccus* species could be distinguished from the two other carotenoid-producing bacteria (*P. marcusii* DSM 11574<sup>T</sup> and *P. carotinifaciens* E-396<sup>T</sup>) by their inability to use seven carbon sources (adonitol, i-erythritol, gentiobiose, β-methylglucoside, D-sorbitol, xylitol and quinic acid). Two carbon sources that were utilized by all five members of the
- 25 new *Paracoccus* species (L-asparagine and L-aspartic acid) were not used for growth by *P. marcusii* DSM 11574<sup>T</sup>.

Table 8. Fatty acid composition of cell membranes of *Paracoccus* sp. strains R-1512, R1534, R114, R-1506, MBIC3966 and three type strains of other *Paracoccus* species, i.e. *P. marcusii* DSM 11574<sup>T</sup>, *P. carotinifaciens* E-396<sup>T</sup> and *P. solventivorans* DSM 6637<sup>T</sup>

Name	Mean % for:	% for:		
	R-1512, R1534, R114, R-1506 and MBIC3966	DSM 11574 <sup>T</sup>	E-396 <sup>T</sup>	DSM 6637 <sup>T</sup>
10:0 3OH	4.9 ± 1.1	6.2	3.4	3.6

- 57 -

Unnamed 11.799	3.6 ± 0.5	4.9	2.8	3.0
Unnamed 15.275	1.5 ± 0.3	2.9	1.1	ND <sup>a</sup>
16:0	0.3 ± 0.2	ND	0.3	0.7
17:1 w8c	ND	ND	0.6	0.8
17:0	0.1 ± 0.1	ND	0.3	1.3
18:1 w7c	80.5 ± 1.8	80.3	84.0	79.0
	Mean % for:	% for:		
Name	R-1512, R1534, R114, R-1506 and MBIC3966	DSM 11574 <sup>T</sup>	E-396 <sup>T</sup>	DSM 6637 <sup>T</sup>
18:0	3.6 ± 0.4	2.6	5.2	6.6
18:0 3OH	0.6 ± 0.4	ND	ND	ND
19:0	ND	ND	ND	0.7
20:1 w7c	0.8 ± 0.2	ND	0.2	2.0
Summed feature 2	2.7 ± 0.4	3.0	2.1	2.6
Summed feature 3	0.7 ± 0.5	ND	0.2	ND
TOTAL	99.3	99.9	100.2	100.3

<sup>a</sup>ND, not detected

**Biochemical tests.** Selected biochemical features were tested using the API 20NE strip (bioMérieux, France). Cells from each of the bacterial strains identified in Table 10 were grown for 24 hours at 28°C on LMG medium 12. Cell suspensions were prepared and strips inoculated according to the instructions of the manufacturer. Strips were incubated at 28°C and results determined after 24 and 48 hours. The results are summarized in Table 10. Of the nine features tested, only one (urease activity) gave a variable response among the five strains of the new *Paracoccus* species. These nine tests did not differentiate between the new *Paracoccus* species and *Paracoccus marcusii* DSM 11574<sup>T</sup> and *P. carotini-faciens* E-396<sup>T</sup>.

Table 9. Utilization of carbon sources for growth by *Paracoccus* spp. strains.

	R1512	R1534	R114	R1506	MBIC 3966	DSM 11574 <sup>T</sup>	E-396 <sup>T</sup>	DSM 6637 <sup>T</sup>

$\alpha$ -Cyclodextrin	-	-	-	-	-	-	-	-
Dextrin	-	-	-	-	-	-	-	-( $\pm$ )
Glycogen	-	-	-	-	-	-	-	-
Tween 40	-	-	-	-	-	-	-(?)	-
Tween 80	-	-	-	-	-	-	-	-
GalNAc	-	-	-	-	-	-	-	-
	R1512	R1534	R114	R1506	MBIC 3966	DSM 11574 <sup>T</sup>	E-396 <sup>T</sup>	DSM 6637 <sup>T</sup>
GlucNAc	-	-	-	-	-	-	-	-(?)
Adonitol	-	-	-	-	-	+	+	-
L-Arabinose	-	-	-	-	-	+	-	+
D-Arabitol	+	+	+	+	$\pm$ (+)	+	+	-
Cellobiose	$\pm$ (+)	$\pm$ (+)	-(?)	-(+)	-( $\pm$ )	+	+	-(+)
i-Erythritol	-	-	-	-	-	+	+	-
D-Fructose	+	+	+	+	-	+	+	+
L-Fucose	-	-	-	-	-	-	+	-
D-Galactose	+	+	+	$\pm$ (+)	$\pm$ (+)	+	+	-( $\pm$ )
Gentiobiose	-	-	-	-	-	+	+	-( $\pm$ )
$\alpha$ -D-Glucose	+	+	+	$\pm$ (+)	-(+)	+	$\pm$ (+)	+
m-Inositol	+	+	+	-(+)	-(+)	+	-( $\pm$ )	-
$\alpha$ -Lactose	+	$\pm$ (+)	-(+)	-(+)	-(+)	+	+	$\pm$ (+)
Lactulose	-( $\pm$ )	-(+)	-(+)	-( $\pm$ )	-	+	+	-(+)
Maltose	+	+	-(+)	-(+)	-( $\pm$ )	+	+	-(+)
D-Mannitol	+	+	+	+	-(+)	+	+	-( $\pm$ )
D-Mannose	+	+	+	+	-( $\pm$ )	+	+	-(+)
D-Melibiose	+	+	+	-(+)	-(+)	+	+	-(?)
$\beta$ -Methylgluc	-	-	-	-	-	+	+	+

D-Psicose	-(+)	±(+)	±(+)	-	-(+)	-	±	-
D-Raffinose	-	-	-	-	-	-(+)	+	-
L-Rhamnose	-	-	-	-	-	-	-	-(?)
D-Sorbitol	-	-	-	-	-	+	+	-
Sucrose	+	+	±(+)	-(+)	-	+	+	+
D-Trehalose	+	+	-(+)	-(+)	-(+)	+	+	+
	R1512	R1534	R114	R1506	MBIC 3966	DSM 11574 <sup>T</sup>	E-396 <sup>T</sup>	DSM 6637 <sup>T</sup>
Turanose	-(+)	-(+)	-	-	-	+	+	+
Xylitol	-	-	-	-	-	+	+	-
Methylpyruvate	±	-	±	-(?)	±	-	+	-(±)
MMSucc	±(+)	±	-(±)	-(+)	-(±)	-(+)	+	-
Acetic acid	-	-	±	-	-	-	-	+
Cis-aconitic acid	-	±	±	-	-	±	-	-
Citric acid	-	±	±	-	-	±	-	-
Formic acid	-	-	-	-	-	-	-	-
GalAlactone	-(±)	-(±)	-(±)	-	-	-	-(±)	-(?)
GalacturonicA	-	-	-	-	-	-(+)	-(±)	-
D-Gluconic acid	+	+	+	-(±)	-(±)	+	+	+
GlucosaminicA	-	-	-	-	-	-	-	-
GlucuronicA	±	+	+	-(±)	-	±(+)	-	-
AHBA	-(±)	-	-(±)	-	-(+)	-	-	-
BHBA	+	+	+	-(±)	±	-(+)	+	+
GHBA	-	-	-	-	-	-	-	-
PHPAA	-	-	-	-	-	-	-	-(+)
Itaconic acid	-	-	-	-	-	-	-	-

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AKBA	-	-	-	-	-	-	-	-(±)
AKGA	-	-	-	-(±)	-(?)	-(±)	-(+)	-(±)
AKVA	-	-	-	-	-	-	-	-
LAME	-	-	-	-	-	-	-	-
Malonic acid	-	-	-	-	-	-	-	-
Propionic acid	-	±	±	-	-	±	+	+
	R1512	R1534	R114	R1506	MBIC 3966	DSM 11574 <sup>T</sup>	E-396 <sup>T</sup>	DSM 6637 <sup>T</sup>
Quinic acid	-	-	-	-	-	+	+	-
SaccA	-(+)	±	-	-(±)	-	-	-	-
Sebacic acid	-(+)	-(+)	-(+)	-(+)	-(±)	-	-(+)	-
Succinic acid	-	-	-	-	-	-(+)	±	-(?)
BromosuccA	-	-	-	-	-	±	-	-
Succinamic acid	-	-	-	-	-	-(+)	-(+)	-
Glucuronamide	-	-	-	-	-(±)	-	-	-
Alaninamide	-	-	-	-	-	-(+)	+	-
D-Alanine	-	-	-(+)	-	-	-	-	-
L-Alanine	+	+	+	+	-	-(+)	+	+
L-Alanyl-glycine	-(+)	-	-(+)	-	-	-	-(+)	-(?)
L-Asparagine	+	+	±(+)	+	±(+)	-	+	-
L-Aspartic acid	+	+	±(+)	-(+)	-(+)	-	+	-
L-Glutamic acid	+	+	+	+	±(+)	-(+)	+	-(+)
GAA	-	-	-	-(±)	-	-	-	-
GGA	-(?)	-	-	-(?)	-	-	-(±)	-
L-Histidine	-	-	-	-	-	-(?)	-	+
HydPro	-	-	-	-	-	-	-	+
L-Leucine	-(±)	-(+)	-(+)	-(+)	-	-(+)	-(?)	-(+)

L-Ornithine	-	-(+)	±(+)	-(±)	-	-	-(+)	-
L-Phenylalanine	-	-	-	-	-	-	-	-
L-Proline	+	+	+	+	-	-(+)	+	+
PyroGluA	+	+	+	+	±(+)	-(+)	+	-
D-Serine	-	-	-	-	-	-	-	-
L-Serine	±	±(+)	-(+)	-(±)	-(+)	-(+)	-(+)	+
	R1512	R1534	R114	R1506	MBIC 3966	DSM 11574 <sup>T</sup>	E-396 <sup>T</sup>	DSM 6637 <sup>T</sup>
L-Threonine	-	-	-	-	-	-(+)	-	-
D,L-Carnitine	-	-	-	-	-	-	-	-
GABA	-	-	-	-	-(+)	-	-(+)	-(+)
Urocanic acid	-	-	-	-	-	-	-	-(+)
Inosine	-	-(±)	-	-	-	-(±)	-(+)	-(+)
Uridine	-	-	-	-	-	-(±)	-(+)	-
Thymidine	-	-	-	-	-	-(±)	-(±)	-
PEA	-	-	-	-	-	-	-	-
Putresceine	-	-	-	-	-	-	-	-
2-Aminoethanol	-	-	-	-	-	-	-	-
2,3-Butanediol	-	-	-	-	-	-	-	-
Glycerol	+	+	+	-(+)	-	+	+	-
GlycP	-	-	-	-	-(±)	-	-	-
Gluc-1-P	-	-	-	-	-	-	-(±)	-
Gluc-6-P	-	-	-	-	-	-	-	-

GalNAc: N-Acetyl-D-galactosamine; GlucNAc: N-Acetyl-D-glucosamine; β-Methylgluc:

β-Methylglucoside; MMSucc: Mono-methylsuccinate; GalAlactone: D-Galactonic acid

lactone; GalacturonicA: D-Galacturonic acid; GlucosaminicA: D-Glucosaminic acid;

GlucuronicA: D-Glucuronic acid; AHBA: α-Hydroxybutyric acid; BHBA: β-Hydroxy-

5 butyric acid; GHBA: γ-Hydroxybutyric acid; PHPAA: p-Hydroxyphenylacetic acid; AKBA:

- $\alpha$ -Ketobutyric acid; AKGA:  $\alpha$ -Ketoglutaric acid; AKVA:  $\alpha$ -Ketovaleric acid; LAME:  
 D,L-Lactic acid methyl ester; SaccA: D-Saccharic acid; BromosuccA: Bromosuccinic acid;  
 GAA: Glycyl-L-aspartic acid; GGA: Glycyl-L-glutamic acid; HydPro: Hydroxy-L-proline;  
 PyroGluA: L-Pyroglutamic acid; GABA:  $\gamma$ -Aminobutyric acid; PEA: Phenylethylamine;  
 5 GlycP: D,L- $\alpha$ -Glycerolphosphate; Gluc-1-P: Glucose-1-phosphate; Gluc-6-P: Glucose-6-  
 phosphate

Table 10. Biochemical features of *Paracoccus* spp. strains: 12 = R1512; 34 = R1534; 14 = R114, 06 = R1506; 66 = MBIC3966; 74 = DSM 11574<sup>T</sup>, 96 = E-396<sup>T</sup>, 37 = DSM 6637<sup>T</sup>

	12	34	14	06	66	74	96	37
Reduction nitrate to nitrite	-	-	-	-	-	-	-	+
Reduction nitrate to nitrogen	-	-	-	-	-	-	-	+
Indole from tryptophan	-	-	-	-	-	-	-	-
Fermentation of glucose	-	-	-	-	-	-	-	-
Arginine hydrolase	-	-	-	-	-	-	-	-
Urease	S / + 5	-	-	S / + 5	+	-	-	-
Esculine hydrolysis <sup>a</sup>	weak	S / + 5	S / + 5	+	+	+	+	+
Gelatine hydrolysis <sup>b</sup>	-	-	-	-	-	-	-	-
$\beta$ -Galactosidase	+	+	+	+	+	+	+	-

<sup>a</sup>:  $\beta$ -glucosidase; <sup>b</sup>: protease; S / + 5: Slow + 5 days

- 10 **Physiological tests.** Several physiological and morphological tests were performed on the five strains of the new *Paracoccus* species, along with *Paracoccus marcusii* DSM 11574<sup>T</sup>, *Paracoccus carotinifaciens* E-396<sup>T</sup> and *Paracoccus solventivorans* DSM 6637<sup>T</sup>. The methods used for each test were as follows.

- Temperature range for growth.* Cells were grown for 24 hours at 28°C on LMG medium 12.  
 15 A cell suspension with a density of between 1-2 McFarland units was prepared in sterile distilled water. From this suspension, 3 drops were transferred onto the agar surface of LMG medium 12. One drop was diluted by streaking, the other 2 drops were left undisturbed. The plates were incubated under aerobic conditions at 10°C, 25°C, 30°C, 33°C, 37°C and 40°C, and checked for growth after 24 hours, 48 hours and 5 days. Growth was  
 20 determined as visual growth (confluent in the drops and as colonies in the streaks with

diluted inoculum) compared to the growth at 30°C (i.e., the "control"). Scoring was done (vs. the control plate) as follows; better growth (++), good (equivalent to the control) growth (+), weaker growth ( $\pm$ ), poor growth ( $\pm$ ), and no growth (-). Results in parentheses are those observed in the streaks if different from the confluent growth in the undisturbed drops.

*Salt tolerance.* Cells were grown for 24 hours at 28°C on LMG medium 12. A cell suspension with a density of between 1-2 McFarland units was prepared in sterile distilled water. From this suspension, 3 drops were transferred onto the agar surface of LMG medium 12 supplemented with NaCl to reach final concentrations of 3%, 6% and 8%. One drop was diluted by streaking, the other 2 drops were left undisturbed. The plates were incubated under aerobic conditions at 28°C and checked for growth after 24 hours, 48 hours and 5 days. Growth was determined as visual growth (confluent in the drops and as colonies in the streaks with diluted inoculum) compared to the growth without added NaCl (control). Scoring was done (vs. the control plate) as follows; better growth (++), good (equivalent to the control) growth (+), weaker growth ( $\pm$ ), poor growth ( $\pm$ ), and no growth (-). Results in parentheses are those observed in the streaks if different from the confluent growth in the undisturbed drops.

*pH Range for growth.* Cells were grown for 24 hours at 28°C in LMG medium 12. A cell suspension with a density of between 1-2 McFarland units was prepared in sterile distilled water. From this suspension, 3 drops were transferred into tubes containing 10 ml liquid LMG medium 12 with modified pH, giving final pH values after autoclaving of pH 6.1, pH 6.3, pH 7.0, pH 7.7, pH 8.1 and pH 9.1. The liquid cultures were incubated aerobically (with shaking) at 28°C. Growth was checked at 24 hours, 48 hours, 3 days and 6 days. Growth was determined as increased turbidity (measured as % transmission using the BIOLOG turbidimeter) compared to growth at pH 7.0 (control). Scoring was done (vs. the control) as follows; better growth (++), good (equivalent to the control) growth (+), weaker growth ( $\pm$ ), poor growth ( $\pm$ ), and no growth (-).

*Starch hydrolysis.* Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A loopful of cells was taken from the plate and transferred as one streak onto the agar surface of LMG medium 12 supplemented with 0.2% soluble starch. Plates were then incubated under aerobic conditions at 28°C. When the strains had developed good growth (after 48 hours), the plate was flooded with lugol solution (0.5% I<sub>2</sub> and 1% KI in distilled water). Hydrolysis was determined as a clear zone alongside the growth (in contrast to the blue color of the agar where starch was not hydrolyzed).



- Denitrification.* Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A loopful of cells was taken from the plate and stabbed into tubes containing semi-solid (0.1% agar) LMG medium 12 supplemented with 1% KNO<sub>3</sub>. The plates were incubated at 28°C for 5 days. Denitrification (N<sub>2</sub> from nitrate) was determined as gas formation alongside the stab.
- Growth under anaerobiosis without electron acceptor added.* Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A loopful of cells was taken from the plate and streaked onto the agar surface of LMG medium 12. The agar plates were incubated under anaerobic conditions (ca. 10% CO<sub>2</sub> + ca. 90% N<sub>2</sub>) at 30°C. Plates were checked for growth after 24 hours and after 5 days. Growth was determined visually and compared to the aerobic (control) condition. Scoring was done (vs. the control) as follows; better growth (++) , good (equivalent to the control) growth (+) , weaker growth (±) , poor growth (±) , and no growth (-).
- Growth under anaerobic conditions with glucose added (fermentation).* Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A loopful of cells was taken from the plate and stabbed into tubes containing the basal agar medium of Hugh and Leifson [J. Bacteriol. 66:24-26 (1953)]. Paraffin oil was added to the top of the medium, and the tubes were incubated at 30°C. Tubes were checked for growth and acid formation after 48 hours and after 5 days. Growth was determined visually. Scoring was done as follows; good growth (+) , poor growth (±) , and no growth (-).
- Growth under anaerobic conditions with KNO<sub>3</sub> as electron acceptor.* Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A loopful of cells was taken from the plate and streaked onto the agar surface of LMG medium 12 supplemented with 0.1% KNO<sub>3</sub>. The plates were incubated under anaerobic conditions (ca. 10% CO<sub>2</sub> + ca. 90% N<sub>2</sub>) at 30°C, and checked for growth after 3 days. Growth was determined as visual growth compared to the aerobic (control) condition. Scoring was done (vs. the control) as follows; better growth (++) , good (equivalent to the control) growth (+) , weaker growth (±) , poor growth (±) , and no growth (-).
- Catalase and oxidase reactions.* Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A positive result for catalase activity was the production of gas bubbles after suspending a colony in one drop of 10% H<sub>2</sub>O<sub>2</sub>. A positive result for oxidase activity was the development of a purple-red color after rubbing a colony on filter paper soaked with 1% tetramethylparaphenylene.

*Colony pigmentation.* Cells were grown for 5 days at 28°C on LMG medium 12. Color of colonies was observed visually.

*Cell morphology and motility.* Cells were grown for 24 hours at 28°C on LMG medium 12. Cell suspensions were made in sterile saline. Cell morphology and motility were observed  
5 microscopically using an Olympus light microscope equipped with phase contrast optics (magnification 1000x).

The results of the physiological and morphological tests are summarized in Table 11. The five strains of the new *Paracoccus* species responded essentially identically in all physiological and morphological tests performed. The tests that gave identical responses for all five  
10 strains of the new *Paracoccus* species and that allowed discrimination of these organisms from *Paracoccus marcusii* DSM 11574<sup>T</sup> and/or *Paracoccus carotinifaciens* E-396<sup>T</sup> were: growth at 40°C, growth with 8% NaCl, growth at pH 9.1, and colony pigmentation.

*Zeaxanthin production in strains R-1512, R1534, R114 and R-1506 strains.* Strains R-1512, R1534, R114, and R-1506 were grown in ME medium, which contains (per liter  
15 distilled water): 5 g glucose, 10 g yeast extract, 10 g tryptone, 30 g NaCl and 5 g MgSO<sub>4</sub>·7H<sub>2</sub>O. The pH of the medium was adjusted to 7.2 with 5N NaOH before sterilizing by autoclaving. All cultures (25-ml volume in 250-ml baffled Erlenmeyer flasks with plastic caps) were grown at 28°C with shaking at 200 rpm. Seed cultures were inoculated from frozen glycerolized stocks and grown overnight. Aliquots were  
20 transferred to the experimental flasks to achieve an initial optical density at 660 nm (OD<sub>660</sub>) of 0.16. Cultures were then grown at 28°C with shaking at 200 rpm. Growth was monitored throughout the cultivation and at 6, 10 (or 15 for strain R114), and 24 hours, an aliquot of the culture was removed for analysis of carotenoids by the method described in Example 1.

25 The doubling times of strains R-1512, R1534 and R-1506 under these conditions were 0.85 hours, 1.15 hours and 1.05 hours, respectively. Strain R114 reproducibly exhibited a bi-phasic growth profile; the doubling time of strain R114 in the initial phase was 1.4 hours while the doubling time in the second phase was 3.2 hours.

Table 12 shows the zeaxanthin production and Specific Formation (zeaxanthin production  
30 normalized to OD<sub>660</sub>) by the *Paracoccus* sp. strains in ME medium. The data are averages of four independent experiments, and within each experiment each strain was tested in duplicate flasks. The improved zeaxanthin production in the classically-derived mutant strains R1534 and R114 compared to the parental strain R-1512 is clearly shown. Zea-

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xanthin production by strain R-1506 was approximately the same as strain R-1512. No other carotenoids were detected in any of the cultures.

Table 11. Physiological characteristics of *Paracoccus* spp. strains: 12 = R1512; 34 = R1534; 14 = R114, 06 = R1506; 66 = MBIC3966; 74 = DSM 11574<sup>T</sup>, 96 = E-396<sup>T</sup>, 37 = DSM 6637<sup>T</sup>

Time [days]	12	34	14	06	66	74	96	37
Growth at 10°C								
1	-	-	-	-	-	-	-	-
5	± (±)	± (±)	± (-)	± (±)	± (-)	± (±)	± (±)	± (±)
Growth at 25°C								
1	+	+	+	+	± (±)	± (±)	± (±)	± (-)
5	+	+	+	+	+	+	+	+
Growth at 30°C								
1	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
Growth at 33°C								
1	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
Growth at 37°C								
1	+	+	± (±)	+	+	± (-)	± (-)	+
5	+	+	+	+	+	± (-)	± (±)	+
Growth at 40°C								
1	+	± (±)	± (-)	± (±)	± (-)	-	-	± (*)
5	+	± (±)	± (-)	+	± (-)	-	-	± (*)
Growth with 3% NaCl								
1	+	+	+	+	+	+	+	±
5	+	+	+	+	+	+	+	+
Growth with 6% NaCl								
1	± (±)	± (±)	± (±)	+	± (±)	± (-)	± (-)	-
5	+	+	+	+	± (*)	± (±)	± (±)	-

Time [days]	12	34	14	06	66	74	96	37
	Growth with 8% NaCl							
1	+(±)	±(±)	±(-)	+(±)	±(±)	-	-	-
5	+	+	+	+	+(*)	±(-)	±(-)	-
	Growth at pH 6.1							
1	+	+	+	+	-	-	-	-
6	+	+	+	+	+	+	+	+
	Growth at pH 6.3							
1	+	+	+	+	+	±	+	±
6	+	+	+	+	+	+	+	+
	Growth at pH 7.0							
1	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+
	Growth at pH 7.7							
1	+	+	+	+	+	±	±	±
6	+	+	+	+	+	+	+	+
	Growth at pH 8.1							
1	+	+	+	+	+	-	-	±
6	+	+	+	+	+	+	+	+
	Growth at pH 9.1							
1	±	+	-	-	+	-	-	-
6	+	+	+	+	+	-	+	+
	Starch hydrolysis							
	-	-	-	-	-	-	-	-
	Denitrification							
	-	-	-	-	-	-	-	+
	Growth in anaerobiosis without electron acceptor added							
	-	-	-	-	-	-	-	-

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	12	34	14	06	66	74	96	37
	Growth in anaerobiosis with glucose added (fermentation)							
	-	-	-	-	-	-	-	±
	Growth in anaerobiosis with KNO <sub>3</sub> added							
	-	-	-	-	-	-	-	-
	Catalase reaction							
	+	+	+	+	+	+	+	+
	Oxidase reaction							
	+	+	+	+	+	+	+	+
	Gram stain							
	-	-	-	-	-	-	-	-
	Motility							
	-	-	-	-	-	-	-	-
	Colony pigmentation							
	Y - O	Y - O	Y - O	Y - O	Y - O	O - P	O - P	P Y
	Cell morphology							
	S to C	S to C	S to C	C	S to C	S	S	S
	Cell dimensions (µm)							
	0.8 x 1.2	0.8 x 1.2	0.8 x 1.2	0.9 x 1.1	0.8 x 1.2 to 1.5	0.8 x 1.5 to 2.0	0.9 x 2.0 to 2.5	0.8 x 1.5 to 2.0

Y - O: yellow-orange; O - P: orange-pink; P Y: pale yellow; S to C: short rod to coccoid; S: short rod; C: coccoid

Table 12. Zeaxanthin production by *Paracoccus* sp. strains R-1512, R1534, R114 and R-1506.

Strain	Time (h)	Zeaxanthin (mg/l)		Specific Formation (mg zeaxanthin/OD <sub>600</sub> )	
		Average	Standard Deviation	Average	Standard Deviation
R-1512	6	0.23	0.10	0.10	0.04
	10	2.05	0.70	0.25	0.08
	24	3.78	0.59	0.38	0.06
R1534	6	0.75	0.10	0.26	0.02
	10	3.45	0.57	0.43	0.07
	24	9.13	0.97	0.95	0.06
R114	6	0.65	0.17	0.86	0.24
	15	7.53	1.12	1.13	0.21
	24	19.7	1.82	2.68	0.20
R-1506	6	0.13	0.06	0.07	0.01
	10	1.35	0.31	0.19	0.04
	24	3.55	0.68	0.38	0.07

**Example 3: IPP Biosynthesis via the Mevalonate Pathway in the Zeaxanthin-Producing *Paracoccus* sp. strain R114.**

5

In order to determine the biosynthetic origin (i.e., the mevalonate or DXP pathway) of isoprenoid precursors in *Paracoccus* sp. strain R114, a "retrobiosynthesis" approach [Eisenreich and Bacher, In: Setlow (ed.), Genetic Engineering, Principles and Methods, Kluwer Academic/Plenum Publishers, New York 22:121-153 (2000)] was taken. This predictive approach for data analysis permits the unequivocal assessment of glucose catabolism from the analysis of a single down-stream natural product. In the present work, this involved growth of the bacterium in media containing various binary mixtures of unlabeled glucose and specific <sup>13</sup>C-labeled glucoses, followed by purification of the zeaxanthin produced and analysis of the labeling patterns by NMR spectroscopy. Details of the methods used and the experimental results are given below.

15

Growth of *Paracoccus* sp. strain R114 for <sup>13</sup>C labeling experiments. Unlabelled D-glucose monohydrate was purchased from Fluka (Milwaukee, WI, USA). [U-<sup>13</sup>C<sub>6</sub>]-D-Glucose was purchased from Isotec (Miamisburg, OH, USA), while [1-<sup>13</sup>C<sub>1</sub>] D-glucose, [2-<sup>13</sup>C<sub>1</sub>] D-glucose and [6-<sup>13</sup>C<sub>1</sub>] D-glucose were from Cambridge Isotope Laboratories (Andover, MA, USA). Yeast extract and peptone (from casein, pancreatically digested) were purchased

20

from EM Science (Gibbstown, NJ, USA). All other salts and solvents were analytical grade and were purchased from standard chemicals suppliers.

All cultures were initiated from frozen cell suspensions (cell density of 12 OD<sub>660</sub> units, 25% glycerol, stored at -70°C). One ml of thawed cell suspension was used to inoculate pre-cultures (500-ml baffled shake flasks) containing 100 ml of 362F/2 medium having the following composition: 30 g/l D-glucose, 10 g/l yeast extract, 10 g/l peptone, 5 g/l NaCl, 2.5 g/l MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.75 g/l (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 0.625 g/l K<sub>2</sub>HPO<sub>4</sub>, 187.5 mg/l CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.2 g/l (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, 15 mg/l ZnSO<sub>4</sub>·7H<sub>2</sub>O, 12.5 mg/l FeCl<sub>3</sub>·6H<sub>2</sub>O, 5 mg/l MnSO<sub>4</sub>·H<sub>2</sub>O, 0.5 mg/l NiSO<sub>4</sub>·6H<sub>2</sub>O, 15 mg/l Na-EDTA and 9.375 µl/l HCl (37% stock solution). The initial pH of the medium was 7.2.

The pre-culture was incubated at 28°C with shaking at 200 rpm for 24 h, after which time the OD<sub>660</sub> was about 22 absorbance units. The main cultures were grown in Bioflo 3000 bioreactors (New Brunswick Scientific, Edison, NJ, USA) containing 362F/2 medium containing the following composition: 30 g/l total D-glucose (see below for ratios of <sup>13</sup>C-labeled:unlabeled glucose), 20 g/l yeast extract, 10 g/l peptone, 10 g/l NaCl, 5 g/l MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.5 g/l (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 1.25 g/l K<sub>2</sub>HPO<sub>4</sub>, 0.4 g/l (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, 375 mg/l CaCl<sub>2</sub>·2H<sub>2</sub>O, 30 mg/l ZnSO<sub>4</sub>·7H<sub>2</sub>O, 25 mg/l FeCl<sub>3</sub>·6H<sub>2</sub>O, 10 mg/l MnSO<sub>4</sub>·H<sub>2</sub>O, 1 mg/l NiSO<sub>4</sub>·6H<sub>2</sub>O, 30 mg/l Na-EDTA and 18.75 µl/l HCl (37% stock solution). The amounts of each <sup>13</sup>C-labeled glucose used (expressed as a percentage of the total 30 g/l glucose in the medium) in four separate experiments were: Condition 1, 4% [U-<sup>13</sup>C<sub>6</sub>] D-glucose; Condition 2, 50% [1-<sup>13</sup>C<sub>1</sub>] D-glucose; Condition 3, 25% [2-<sup>13</sup>C<sub>1</sub>] D-glucose + 1% [U-<sup>13</sup>C<sub>6</sub>] D-glucose; Condition 4, 25% [6-<sup>13</sup>C<sub>1</sub>] D-glucose + 1% [U-<sup>13</sup>C<sub>6</sub>] D-glucose. A control with only unlabeled glucose was also included. For Conditions 1 and 2 (and the unlabeled control), the culture volume was 2 l, while the culture volume for Conditions 3 and 4 was 1 l. The bioreactors were inoculated with pre-culture (20 ml/l initial volume) and cultivation proceeded for 22-24 hours, at which time no glucose was left in the medium. Cultivation conditions were: 28°C, pH 7.2 (controlled with 25% H<sub>3</sub>PO<sub>4</sub> and 28% NH<sub>4</sub>OH), dissolved oxygen controlled (in a cascade with agitation) at a minimum of 40%, agitation rate and aeration rate 300 rpm (minimum) and 1 vvm, respectively.

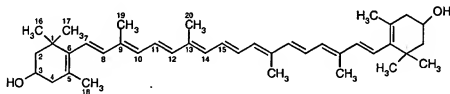
Purification of zeaxanthin. At the end of the cultivations, the cultures were cooled down to 15°C. Five hundred ml of absolute ethanol was added per liter of culture and stirring was continued at 100 rpm for 20 min. The treated culture was centrifuged for 20 min. at 5000 x g, and the supernatant was discarded. The wet pellet was then extracted with 5 volumes of THF for 20 min. with stirring. The extracted mixture was centrifuged, the



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supernatant saved and the resulting pellet extracted a second time with 1 volume THF under the same conditions and again centrifuged. The supernatants (extracts) were combined and concentrated to 50 ml by rotary evaporation. Five milliliters of hexane were added to the concentrated THF solution. After mixing, the system formed an emulsion that could be separated by centrifugation. The aqueous phase was collected, diluted with an equal volume of saturated NaCl solution and re-extracted with dichloromethane. The dichloromethane phase was collected and combined with the THF/hexane phase. The mixture of organic extracts was concentrated again in a rotary evaporator to remove dichloromethane. The solution was then applied to a silica gel column and eluted with a mixture of n-hexane and ether (1:1). A small light yellow band eluted first and was discarded. The main zeaxanthin product eluted in a broad band that moved slowly in the column. About 2 liters of solvent was needed to elute the main band completely. The eluate was collected in a round-bottomed flask and the solvent was removed by rotary evaporation at 40°C. The residue was dissolved in a small amount of dichloroethane at 40°C and the solution was then allowed to cool slowly. Hexane was added to the mixture dropwise until a turbidity was observed. The crystallization was complete within 48 hours at 4°C. The crystals were collected on a paper filter, washed with cold methanol and dried under vacuum.

NMR studies. Zeaxanthin was analyzed by NMR spectroscopy. For reference, the chemical structure of zeaxanthin is illustrated in the following formula



<sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded at 500.13 MHz and 125.6 MHz, respectively, with a Bruker DRX 500 spectrometer. Acquisition and processing parameters for one-dimensional experiments and two-dimensional INADEQUATE experiments were according to standard Bruker software (XWINNMR). The solvent was deuterated chloroform. The chemical shifts were referenced to solvent signals.

<sup>13</sup>C NMR spectra of the isotope labeled zeaxanthin samples and of the zeaxanthin sample at natural <sup>13</sup>C abundance were recorded under the same experimental conditions. Integrals were determined for every <sup>13</sup>C NMR signal, and the signal integral for each respective carbon atom in the labeled compound was referenced to that of the natural abundance material, thus affording relative <sup>13</sup>C abundances for each position in the labeled

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molecular species. The relative abundances were then converted into absolute abundances from  $^{13}\text{C}$  coupling satellites in the  $^1\text{H}$  NMR signal of H-18 at 1.71 ppm. In the  $^{13}\text{C}$  NMR spectrum of the multiply-labeled zeaxanthin sample each satellite was integrated separately. The integral of each respective satellite pair was then referenced to the total signal integral of a given carbon atom. Zeaxanthin comprises a total of eight isoprenoid moieties (2 DMAPP units and 6 IPP units); only 20  $^{13}\text{C}$  NMR signals are observed due to chemical shift degeneracy.

In the experiment with the mixture of [ $\text{U-}^{13}\text{C}_6$ ] glucose and unlabeled glucose (1:7.5; w/w), all carbon atoms of zeaxanthin were labeled and showed satellites due to  $^{13}\text{C}^{13}\text{C}$  couplings (Table 13). The signals of 4 carbon atoms have intense satellites due to  $^{13}\text{C}^{13}\text{C}$  couplings ( $61.2 \pm 0.6\%$  in the global NMR signal intensity of a given atom, Table 13). The signal accounting for the methyl atoms C-17/C-17' displayed only weak  $^{13}\text{C}$ -coupled satellites at a relative intensity of 6%. The central signals represent material derived from unlabeled glucose. The signals showed no evidence of long-range coupling. Carbon connectivity was easily gleaned from  $^{13}\text{C}^{13}\text{C}$  coupling constants (Table 13) and from two-dimensional INADEQUATE experiments.

Three of the carbon atoms acquired label from [ $6\text{-}^{13}\text{C}_1$ ] glucose. The other two carbons were labeled from [ $2\text{-}^{13}\text{C}_1$ ] glucose. No significant amounts of label were contributed to zeaxanthin by [ $1\text{-}^{13}\text{C}_1$ ] glucose.

The  $^{13}\text{C}$  abundance for all non-isochronous carbon atoms was determined by comparison with spectra of unlabeled zeaxanthin and by evaluation of the  $^1\text{H}^{13}\text{C}$  coupling satellites in  $^1\text{H}$  NMR spectra (Table 13). The fraction of jointly transferred carbon atom pairs in the experiment with [ $\text{U-}^{13}\text{C}_6$ ] glucose was determined by integration of the coupling satellites.

The labeling patterns of the IPP building block can be reconstructed accurately as shown by the standard deviations found for the reconstructed IPP precursor. The reconstructed labeling patterns of DMAPP and IPP were identical within the experimental limits.

Table 13. NMR results for  $^{13}\text{C}$  labeled zeaxanthin produced by *Paracoccus* sp. strain R114 supplied with  $^{13}\text{C}$  labeled glucoses.

Position	$\delta - ^{13}\text{C}$ ppm	$J_{\text{CC}}, \text{Hz}$	$^{13}\text{C}$ -labeled glucose precursor				
			[1- $^{13}\text{C}$ ]-	[2- $^{13}\text{C}$ ]-	[6- $^{13}\text{C}$ ]-	[U- $^{13}\text{C}_6$ ]glucose	
	134.08	44.2 (18, 18')	% $^{13}\text{C}$	% $^{13}\text{C}$	% $^{13}\text{C}$	% $^{13}\text{C}$	% $^{13}\text{C}^{13}\text{C}$
1, 1'	37.13	36.0 (16, 16')	1.10	10.71	2.22	3.47	61.2
2, 2'	48.46	35.8 (3, 3')	1.20	2.58	10.27	3.65	61.1
3, 3'	65.10	35.8 (2, 2')	1.12	12.47	2.38	3.64	60.4
4, 4'	42.57	37.1	1.27	2.59	10.63	3.89	8.4
5, 5'	126.17	44.2 (18, 18')	1.14	12.45	3.19	3.68	61.1
6, 6'	137.77	56.4 (7, 7')	1.30	2.15	9.98	3.60	60.4
7, 7'	125.59	56.2 (6, 6')	1.12	10.11	2.82	4.09	61.4
8, 8'	138.50	71.6, 55.7	1.28	2.24	9.95	3.92	4.3, 5.0
9, 9'	135.69	43.1 (19, 19')	1.12	9.53	2.95	3.84	61.7
10, 10'	131.31	59.7 (11, 11')	1.21	3.18	9.61	3.80	61.1
11, 11'	124.93	59.7 (10, 10')	1.10	8.79	2.70	4.02	61.0
12, 12'	137.57	70.5	1.20	2.01	8.80	3.59	5.1
13, 13'	136.48	43.1 (20, 20')	1.12	9.86	3.59	3.93	61.4
14, 14'	132.60	60.4 (15, 15')	1.21	2.83	10.51	3.77	59.5
15, 15'	130.08	60.4 (14, 14')	1.12	9.18	3.33	4.02	61.2
16, 16'	30.26	36.3 (1, 1')	1.27	3.19	12.31	3.91	62.0
17, 17'	28.73	34.9 (1, 1')	1.30	3.43	12.31	3.88	6.0
18, 18'	21.62	44.2 (5, 5')	1.27	3.01	11.66	3.70	62.0
19, 19'	12.82	43.1 (9, 9')	1.29	3.12	11.64	3.86	62.3
20, 20'	12.75	42.9 (13, 13')	1.33	3.21	11.99	3.75	62.1

- The experimental labeling patterns determined above can be compared with various pre-
- 5 predictions, taking into account not only the mevalonate pathway vs. the DXP pathway for isoprenoid biosynthesis, but also different pathways of glucose metabolism. Eubacteria typically utilize glucose primarily via the glycolytic pathway or via the Entner-Doudoroff pathway. Glycolysis generates two triose phosphate molecules from glucose. The C-1 and C-6 of glucose are both diverted to the 3-position of the triose phosphates produced
- 10 during glycolysis. On the other hand, in the Entner-Doudoroff pathway, glucose is converted to a mixture of glyceraldehyde 3-phosphate and pyruvate. The C-1 of glucose is

exclusively diverted to C-1 of pyruvate, and the C-6 of glucose is exclusively diverted to C-3 of glyceraldehyde 3-phosphate.

Intermediates and products of the glycolytic and Entner-Doudoroff pathways serve as starting material for both isoprenoid biosynthetic pathways. With regard to the mevalonate pathway, pyruvate as well as triose phosphate can be converted to the precursor acetyl-CoA. Glucose catabolism via the glycolytic pathway diverts label from C-1 as well as C-6 of glucose to the methyl group of acetyl-CoA. Glucose catabolism via the Entner-Doudoroff pathway results in loss of C-1 from glucose during the transformation of pyruvate to acetyl-CoA.

- 10 The experimentally observed enrichment and  $^{13}\text{C}/^{13}\text{C}$  coupling patterns of the zeaxanthin produced by *Paracoccus* sp. strain R114 were in perfect agreement with the labeling pattern required for zeaxanthin biosynthesis by the combination of the Entner-Doudoroff pathway and the mevalonate pathway. If both the glycolytic and Entner-Doudoroff pathways had been simultaneously operative under the experimental conditions used, at least some label from  $[1-^{13}\text{C}_1]$  glucose should have been contributed to the zeaxanthin. Furthermore, the mevalonate pathway can at best contribute blocks of two carbon atoms to terpenoids, while in the DXP pathway three carbon units can be delivered to isoprenoids via triose phosphate precursors. Although such three-carbon blocks become separated by the rearrangement involved in the DXP pathway, blocks of three labeled carbon atoms can still be recognized via long-range coupling. Corresponding  $^{13}\text{C}-^{13}\text{C}$  long-range couplings have been observed in the biosynthesis of the carotenoid lutein from  $[2,3,4,5-^{13}\text{C}_4]$  1-deoxy-D-xylulose by cultured plant cells (*Cantharantus roseus*) [Arigoni et al., Proc. Nat. Acad. Sci. 94:10600-10605 (1997)]. No such long-range coupling was observed in the present experiments with zeaxanthin produced by *Paracoccus* sp. strain R114.

- It should be noted that while the results presented here confirm isoprenoid production in *Paracoccus* sp. strain R114 via the mevalonate pathway, and indicate that, under the growth conditions used, there was little or no glucose metabolism via glycolysis, they do not rule out the possibility of some metabolism of glucose via the pentose phosphate pathway in addition to the Entner-Doudoroff pathway. Quantitative determination of glucose metabolism via the latter two pathways could be obtained by analysis of labeling patterns of pyruvate-derived amino acids (as was done for *Paracoccus denitrificans* [Dunstan et al., Biomedical and Environ. Mass Spectrometry 19:369-381 (1990)]).

Example 4: Cloning and Sequencing of the Genes Encoding IPP Isomerase and the Enzymes of the Mevalonate Pathway from *Paracoccus* sp. strain R114.

Culture conditions. *Paracoccus* sp. strain R114 was grown at 28°C in F-medium (10 g/l tryptone, 10 g/l yeast extract, 30 g/l NaCl, 10 g/l D-glucose, 5 g/l  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , pH 7.0) or in the pre-culture medium described in Example 3 above. Liquid cultures were grown in a rotary shaker at 200 rpm.

- Isolation of genomic DNA. A 600-ml culture of *Paracoccus* sp. strain R114 was centrifuged for 10 minutes at 10,000 x g at 4°C and the pellet was washed once with 200 ml lysis buffer (0.1M NaCl, 50mM EDTA, 10mM Tris-HCl, pH 7.5) and once with 100 ml lysis buffer.
- 10 The final pellet was resuspended in 20 ml lysis buffer containing 50 mg lysozyme and 1 mg RNase A (DNase free). After incubation for 15 minutes at 37°C, 1.5 ml of 20% sodium N-lauroyl-sarcosinate and 2.25 mg of proteinase K were added. After incubation at 50°C for 30-60 minutes, the lysate was extracted with one volume of buffer-saturated phenol, pH 7.5-7.8 (LifeTechnologies, Rockville, MD, USA) by gentle but thorough mixing.
- 15 The emulsion was centrifuged for 20 minutes at 30,000 x g and the aqueous phase was re-extracted with phenol. The phases were separated as before and the aqueous phase was extracted twice with one volume phenol:chloroform (1:1). At this step centrifugation for 20 minutes at 3,200 x g in a swinging bucket rotor was sufficient to obtain satisfactory phase separation. After a final extraction with one volume of chloroform, 0.1 volume 3M sodium-acetate (pH 5.2) was added and the solution was overlaid with 2 volumes ice-cold ethanol. The precipitated DNA was spooled with a glass-rod, soaked in 70% ethanol for 5 minutes, rinsed with chloroform and then air dried for 5-10 minutes. The DNA was re-suspended overnight in 5 ml TE (10mM Tris-HCl, pH 7.5, 1mM EDTA). Since the solution was yellow due to traces of zeaxanthin, the organic extractions and the spooling were
- 25 repeated as above to obtain a clear preparation.

Isolation of  $\lambda$ -DNA: The Qiagen® Lambda Kit (Qiagen, Hilden, Germany) was used following the manufacturer's instructions.

- Polymerase chain reaction (PCR): Oligonucleotides were purchased from LifeTechnologies (Rockville, MD, USA). PCR was performed in a GeneAmp® PCR system 9700 (PE Applied Biosystems, Foster City, CA, USA) using the GC-rich PCR system (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturers instructions. Typically, the  $\text{MgCl}_2$  concentration used was 1.5mM and the resolution solution was added to 1M final concentration.
- 30

- DNA Labeling and detection: The PCR DIG Probe Synthesis Kit and the DIG Luminescent Detection Kit were used for DNA labeling and detection, respectively (both obtained from Roche Molecular Biochemicals, Mannheim, Germany)
- 35

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DNA sequencing: Sequencing reactions were performed using the BigDye<sup>®</sup> DNA sequencing kit (PE Applied Biosystems, Foster City, CA, USA) according to the manufacturers instructions. Sequencing reactions were purified on DyeEx<sup>™</sup> spin columns (Qiagen, Hilden, Germany) and fragment separation and detection was done with an ABI Prism<sup>™</sup> 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

$\lambda$ -library: A custom made library with partially *Sau3AI* digested *Paracoccus* sp. strain R114 DNA in lambda *FLX<sup>®</sup> II* was purchased from Stratagene (La Jolla, CA, USA).

Cloning, sequencing and characterization of the mevalonate pathway gene cluster from *Paracoccus* sp. strain R114. One of the enzymes of the mevalonate pathway, mevalonate diphosphate decarboxylase, contains highly conserved regions spanning several amino acids. Three such regions were chosen from an alignment of all available eubacterial mevalonate diphosphate decarboxylases and oligonucleotides were designed using the preferred codon usage found in the carotenoid gene cluster of *Paracoccus* sp. strain R1534 (Table 14).

The oligonucleotides designed from two homology regions are shown in Table 15. To reduce the degree of degeneracy, sets of oligonucleotides were designed from each peptide. For instance, oligonucleotides mvd-103a-d differ only in the third nucleotide from the 3' end, each accounting for one possible codon for glycine (GGA, although rarely used, was included because of the close proximity to the 3' end). Alternate amino acids were accounted for by designing oligonucleotides to both residues, e.g. oligonucleotides mvd-101a and mvd-101b are specific for leucine or isoleucine, respectively, in the second position of peptide 1 (Table 15). PCR with oligonucleotides mvd-101 and mvd-104 or mvd-106, using *Paracoccus* sp. strain 114 DNA as template, gave a product of the expected size. The PCR product was cloned in the vector pCR<sup>®</sup>2.1-TOPO (Invitrogen, Carlsbad, CA, USA) and sequenced. The cloned fragment was used as a probe for a Southern analysis of *Paracoccus* sp. strain R114 DNA and was found to hybridize to a *Bam*HI-*Sal*I fragment of about 950 bp. *Paracoccus* sp. strain R114 DNA was cut with *Bam*HI and *Sal*I and the fragments were separated by agarose gel electrophoresis. The region around 950 bp was isolated and cloned in the vector pUC19. This partial library was then screened using the *mvd*-PCR fragment as a probe and the insert of a positive clone was sequenced. In parallel, a  $\lambda$ -library prepared from *Paracoccus* sp. strain R114 DNA was screened using the *mvd*-PCR fragment as a probe. DNA was isolated from two positive  $\lambda$ -clones and cut with *Bam*HI and *Sal*I or *Eco*RI and *Sal*I. A number of the restriction fragments were isolated and cloned in the vector pUC19. Several of the fragments contained sequences homologous to genes encoding proteins of the mevalonate pathway. The clones connecting these individual sequences were obtained by PCR with primers derived from the sequences of

- the cloned restriction fragments using the DNA of the  $\lambda$ -clones as template. The assembled sequence from all fragments (SEQ ID NO:42, 44, 46, 48, 50, and 52) and the sequences of the encoded proteins are shown in the Sequence Listing (SEQ ID Nos:43, 45, 47, 49, 51, and 53). Due to a limitation of the PatentIn Program, operons with overlapping
- 5 genes cannot be shown as a single sequence. Thus, for each gene in the mevalonate operon, the entire nucleotide sequence of the operon is repeated for each gene. Accordingly, SEQ ID Nos:42, 44, 46, 48, 50, and 52 are identical. For purposes of the present invention, we use SEQ ID NO:42 to refer to the nucleotide sequence of the mevalonate operon.
- 10 The arrangement of the mevalonate pathway genes in the *Paracoccus* sp. strain R114 is unique when compared to known mevalonate gene clusters of other bacteria. Besides *Paracoccus* sp. strain R114, only *Borrelia burgdorferi* and *Streptomyces* sp. strain CL190 (Takagi et al., supra) have all mevalonate genes in a single operon (Wilding et al., supra). In *Streptococcus pyrogenes* all mevalonate genes are clustered in a single locus but they are
- 15 grouped in two operons. All other species have two loci with the two kinases and the mevalonate diphosphate decarboxylase grouped in one operon and the HMG-CoA synthase and the HMG-CoA reductase on a second locus, either forming an operon (in *Streptococcus pneumoniae*) or as separate transcription units. All species except the members of *Staphylococcus* have an additional gene linked with the mevalonate cluster,
- 20 which was recently identified as an IPP isomerase (*idi* gene in *Streptomyces* sp. strain CL190) (Kaneda et al., supra). The two *Enterococcus* species and *Staphylococcus haemolyticus* have an acetyl-CoA acetyltransferase gene linked with the HMG-CoA reductase gene. In the *Enterococcus* species the latter two genes are fused.

Table 14: Codon usage in *Paracoccus* sp. strain R1534 carotenoid (crt) genes

Amino acid	Codon	Number used	% Used
A - Ala	GCT	3	1.4
	GCC	96	46.2
	GCA	15	7.2
	GCG	94	45.2
C - Cys	TGT	0	0.0
	TGC	15	100.0
D - Asp	GAT	46	38.0
	GAC	75	62.0
E - Glu	GAA	17	25.4
	GAG	50	74.6
F - Phe	TTT	3	5.6
	TTC	51	94.4
G - Gly	GGT	16	10.8
	GGC	87	58.8
	GGA	5	3.4
	GGG	40	27.0
H - His	CAT	30	56.6
	CAC	23	43.4
I - Ile	ATT	5	6.4
	ATC	72	92.3
	ATA	1	1.3
K - Lys	AAA	4	14.3
	AAG	24	85.7
L - Leu	TTA	0	0.0
	TTG	5	2.9
	CTT	15	8.7
	CTC	11	6.4
	CTA	1	0.6
	CTG	140	81.4
M - Met	ATG	49	100.0
N - Asn	AAT	4	20.0
	AAC	16	80.0



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Amino acid	Codon	Number used	% Used
P - Pro	CCT	2	2.3
	CCC	41	47.7
	CCA	3	3.5
	CCG	40	46.5
Q - Gln	CAA	6	11.3
	CAG	47	88.7
R - Arg	CGT	11	7.3
	CGC	103	68.2
	CGA	2	1.3
	CGG	26	17.2
	AGA	2	1.3
	AGG	7	4.6
S - Ser	TCT	1	1.1
	TCC	17	19.5
	TCA	0	0.0
	TCG	39	44.8
	AGT	2	2.3
	AGC	28	32.2
T - Thr	ACT	2	2.7
	ACC	36	48.9
	ACA	4	5.3
	ACG	33	44.0
V - Val	GTT	6	5.7
	GTC	61	57.5
	GTA	1	0.9
	GTG	38	35.8
W - Trp	TGG	27	100.0
Y - Tyr	TAT	28	62.2
	TAC	17	37.8

Table 15: Oligonucleotides designed from two conserved bacterial Mvd peptides.

		SEQ a
Peptide 1	AlaLeuIleLysTyrTrpGlyLys Ile <sup>2</sup>	23
Nucleotide sequence <sup>1</sup>	CCSCTGATCAARTAYTGGGGBAARATC	24
Oligonucleotide mvd-101a (5' - 3')	GCSCTGATCAARTAYTGGGG	25
Oligonucleotide mvd-101b (5' - 3')	GCSATCATCAARTAYTGGGG	26
Oligonucleotide mvd-103a (5' - 3')	ATCAARTAYTGGGGTAA	27
Oligonucleotide mvd-103b (5' - 3')	ATCAARTAYTGGGGCAA	28
Oligonucleotide mvd-103c (5' - 3')	ATCAARTAYTGGGGGAA	29
Oligonucleotide mvd-103d (5' - 3')	ATCAARTAYTGGGGAAA	30
Peptide 2	ThrMetAspAlaGlyProAsnVal Gln <sup>2</sup>	31
Nucleotide sequence <sup>1</sup> (5'-3')	ACSATGGAYGCSGGBCCSAAYGTS CAR	32
Complement (3'-5')	TGSTACCTRCGSCCVGGSTTRCAS GTY	33
Oligonucleotide mvd-104a (3' - 5')	TGGTACCTACGSCCVGG	34
Oligonucleotide mvd-104b (3' - 5')	TGGTACCTGCGSCCVGG	35
Oligonucleotide mvd-104c (3' - 5')	TGCTACCTACGSCCVGG	36
Oligonucleotide mvd-104d (3' - 5')	TGCTACCTGCGSCCVGG	37
Oligonucleotide mvd-106a (3' - 5')	TACCTACGSCCVGGSTTRCA	38
Oligonucleotide mvd-106b (3' - 5')	TACCTGCGSCCVGGSTTRCA	39
Oligonucleotide mvd-106c (3' - 5')	TACCTACGSCCVGGSGTYCA	40
Oligonucleotide mvd-106d (3' - 5')	TACCTGCGSCCVGGSGTYCA	41

<sup>a</sup>: SEQ ID NO:<sup>1</sup> using the preferred codons of *Paracoccus* sp. strain R1534, see table 1<sup>2</sup> alternate amino acid present in some enzyme

5 S = C or G; R = A or G; Y = C or T; B = C or G or T; V = A or C or G

The genes of the mevalonate operon from *Paracoccus* sp. strain R114 were identified by homology of the gene products to proteins in general databases. An amino acid alignment of the HMG-CoA reductase from *Paracoccus* sp. strain R114 (SEQ ID NO:43) was performed with bacterial class I HMG-CoA reductases of *Streptomyces* sp. Strain CL190 (SEQ

- ID NO:54), *S. griseolosporeus* (SEQ ID NO:55), and *Streptomyces* sp. strain KO-3899 (SEQ ID NO:56). EMBL/GenBank/DBJ database accession numbers are q9zn4 for *Streptomyces* sp. strain CL190, q9zn1 for *S. griseolosporeus* and q9zn0 for *Streptomyces* sp. strain KO-3899. There are two classes of HMG-CoA reductases [Bochar et al., Mol. Genet. Metab. 66:122-127 (1999); Boucher et al., Mol. Microbiol. 37:703-716 (2000)]. Eubacterial HMG-CoA reductases are generally of class II, whereas class I enzymes are found in eukaryotes and archaea. The *Streptomyces* and the *Paracoccus* HMG-CoA reductases together with the enzyme from *Vibrio cholerae* are the only eubacterial HMG-CoA reductases of class I known so far.
- 10 An amino acid alignment of isopentenyl diphosphate isomerase (IPP isomerase) (*idi*) from *Paracoccus* sp. strain R114 (SEQ ID NO:45) was performed with close homologs found in the EMBL database, i.e. *Erwinia herbicola* (Q01335) (SEQ ID NO:57), *Borrelia burgdorferi* (O51627) (SEQ ID NO:58), *Synechocystis* sp. PCC 6803 (P74287) (SEQ ID NO:59), *Streptomyces* sp. CL190 (Q9KWG2) (SEQ ID NO:60), *Streptomyces griseolosporeus* (Q9KWF6) (SEQ ID NO:61), *Sulfolobus solfataricus* (P95997) (SEQ ID NO:62), *Rickettsia prowasekii* (Q9ZD90) (SEQ ID NO:63), *Deinococcus radiodurans* (Q9RVE2) (SEQ ID NO:64), *Aeropyrum pernix* (Q9YB30) (SEQ ID NO:65), *Halobacterium* sp. NRC-1 (O54623) (SEQ ID NO:66), *Archaeoglobus fulgidus* (O27997) (SEQ ID NO:67), *Pyrococcus abyssi* (Q9UZS9) (SEQ ID NO:68), *Pyrococcus horikoshii* (O58893) (SEQ ID NO:69),
- 20 *Methanobacterium thermoautotrophicum* (O26154) (SEQ ID NO:70), *Methanococcus jannaschii* (Q58272) (SEQ ID NO:71), *Thermoplasma acidophilum* (CAC11250) (SEQ ID NO:72) and *Leishmania major* (Q9NDJ5) (SEQ ID NO:73). EMBL/GenBank/DBJ database accession numbers are given after the organism's name in parentheses. The first nine sequences are from eubacteria and the next eight sequences are from archaea. Interestingly,
- 25 one eukaryotic species, the protozoan parasite *Leishmania major* (SEQ ID NO:73), also has a protein that is highly homologous. This is unexpected because other eukaryotes have a different *idi*, designated type 1 (Kaneda et al., supra). A conserved hypothetical protein from *Bacillus subtilis*, YpgA, also has substantial homology but is considerably smaller than the type 2 *idi*'s.
- 30 An amino acid alignment of bacterial HMG-CoA synthase from *Paracoccus* sp. strain R114 (SEQ ID NO:47) was performed with close homologs found in the EMBL database, i.e. *Streptococcus pneumoniae* (AAG02453) (SEQ ID NO:74), *Streptococcus pyogenes* (AAG02448) (SEQ ID NO:75), *Enterococcus faecalis* (AAG02438) (SEQ ID NO:76), *Enterococcus faecium* (AAG02443) (SEQ ID NO:77), *Staphylococcus haemolyticus* (AAG02427) (SEQ ID NO:78), *Staphylococcus epidermis* (AAG02433) (SEQ ID NO:79),

*Staphylococcus aureus* (AAG02422) (SEQ ID NO:80), *Staphylococcus carnosus* (Q9ZB67) (SEQ ID NO:81), *Streptomyces* sp. CL190 (Q9KWG1) (SEQ ID NO:82), *Streptomyces griseolosporeus* (Q9KWF5) (SEQ ID NO:83) and *Borrelia burgdorferi* (051626) (SEQ ID NO:84). EMBL/GenBank/DDBJ database accession numbers are given after each

5 organism's name in parentheses. The first 43 amino acids of the sequence from *Streptomyces griseolosporeus* are missing in the database version.

An amino acid alignment of bacterial mevalonate diphosphate decarboxylase from *Paracoccus* sp. strain R114 (SEQ ID NO:53) was performed with the orthologous proteins from other bacteria, i.e. *Streptococcus pneumoniae* (AAG02456) (SEQ ID NO:85), *Streptococcus*

10 *pyrogenes* (AAG02451) (SEQ ID NO:86), *Enterococcus faecalis* (AAG02441) (SEQ ID NO:87), *Enterococcus faecium* (AAG02446) (SEQ ID NO:88), *Staphylococcus haemolyticus* (AAG02431) (SEQ ID NO:89), *Staphylococcus epidermis* (AAG02436) (SEQ ID NO:90), *Staphylococcus aureus* (AAG02425) (SEQ ID NO:91), *Streptomyces* sp. CL190 (Q9KWG4) (SEQ ID NO:92), *Streptomyces griseolosporeus* (Q9KWF8) (SEQ ID NO:93) and *Borrelia*

15 *burgdorferi* (051629) (SEQ ID NO:94). EMBL/GenBank/DDBJ database accession numbers are given after each organism's name in parentheses.

Two proteins from *Myxococcus xanthus*, Tac and Taf (database accession numbers q9xb06 and q9xb03, respectively) and a protein from *B. subtilis*, PksG, a putative polyketide biosynthesis protein (database accession number p40830), have substantial homology to the

20 *Paracoccus* sp. strain R114 HMG-CoA synthase. The homology between the *Paracoccus* sp. strain R114 HMG-CoA synthase and the Tac and Taf proteins of the *M. xanthus* is greater than the homology between the HMG-CoA synthases from *Paracoccus* sp. strain R114 and eukaryotes. The bacterial HMG-CoA synthases and the bacterial mevalonate diphosphate decarboxylases share substantial homology with their eukaryotic orthologs. Archaeal

25 HMG-CoA synthases form a more distantly related group of enzymes (Wilding et al., supra) and no mevalonate diphosphate decarboxylase orthologs are found in archaea [Smit and Mushegian, Genome Res. 10:1468-1484 (2000)].

Alignments of the mevalonate kinase (Mvk) (SEQ ID NO:49) and the phosphomevalonate kinase (Pmk) (SEQ ID NO:51) from *Paracoccus* sp. strain R114 were performed to the

30 orthologous proteins from other bacteria, i.e. *Streptococcus pneumoniae* (AAG02455) (SEQ ID NO:95), *Streptococcus pyrogenes* (AAG02450) (SEQ ID NO:96), *Enterococcus faecalis* (AAG02440) (SEQ ID NO:97), *Enterococcus faecium* (AAG02445) (SEQ ID NO:98), *Staphylococcus haemolyticus* (AAG02430) (SEQ ID NO:99), *Staphylococcus epidermis* (AAG02435) (SEQ ID NO:100), *Staphylococcus aureus* (AAG02424) (SEQ ID NO:101),

35 *Streptomyces* sp. CL190 (Q9KWG5) (SEQ ID NO:102), *Streptomyces griseolosporeus*

- (Q9KWF9) (SEQ ID NO:103) and *Borrelia burgdorferi* (051631) (SEQ ID NO:104) (Mvk); and *Streptococcus pneumoniae* (AAG02457) (SEQ ID NO:105), *Streptococcus pyrogenes* (AAG02452) (SEQ ID NO:106), *Enterococcus faecalis* (AAG02442) (SEQ ID NO:107), *Enterococcus faecium* (AAG02447) (SEQ ID NO:108), *Staphylococcus haemolyticus*
- 5 (AAG02432) (SEQ ID NO:109), *Staphylococcus epidermis* (AAG02437) (SEQ ID NO:110), *Staphylococcus aureus* (AAG02426) (SEQ ID NO:111), *Streptomyces* sp. CL190 (Q9KWG3) (SEQ ID NO:112), *Streptomyces griseolosporeus* (Q9KWF7) (SEQ ID NO:113) and *Borrelia burgdorferi* (051630) (SEQ ID NO:114) (Pmk). EMBL/GenBank/DDBJ database accession numbers are given after each organism's name in parentheses.
- 10 There is much less homology among the bacterial kinases than among the bacterial orthologs of the other enzymes of the mevalonate pathway. The mevalonate kinase from *Paracoccus* sp. strain R114 (SEQ ID NO:49) has a 37 amino acid insert in the amino-terminal region, which is lacking in other mevalonate kinases. Together with the bacterial Mvk's some archaeal enzymes, e.g. from *Archaeoglobus fulgidus*, *Methanobacterium thermoautotrophicum* and *Pyrococcus abyssi*, are among the best homologues to the Mvk from *Paracoccus* sp. strain R114. The homology among bacterial phosphomevalonate kinases is even
- 15 weaker than the homology among the bacterial mevalonate kinases. The proteins with the best homologies to the Pmk from *Paracoccus* sp. strain R114 (SEQ ID NO:51) are Mvk's from archaea, e.g. *Aeropyrum pernix*, *Pyrococcus horikoshii*, *M. thermoautotrophicum*, *P. abyssi* and *A. fulgidus*. Since no Pmk's are found in archaea (Smit and Mushegian, supra),
- 20 this suggests that the same kinase might perform both phosphorylations.

Example 5: Over-expression of the Mevalonate Pathway Genes and the *idi* Gene from *Paracoccus* sp. strain R114 in *E. coli*

Cloning and expression of the mevalonate operon in *E. coli*. A  $\lambda$  clone, designated clone

- 25 16, from the *Paracoccus* sp. strain R114  $\lambda$  library (see Example 4) was used as a template for PCR amplification of the entire mevalonate operon. Primers Mevop-2020 and Mevop-9027 (Table 16) were used for PCR.

Table 16. Primers used for amplification of mevalonate operon from *Paracoccus* sp. strain R114.

Primer	Sequence (5'→3')
Mevop-2020	GGGCAAGCTTGTCCACGGCAGCAACCAAGCA (SEQ ID NO:115)
Mevop-9027	CGTAATCCGCGGCCGCGTTTCACGCGCGTC (SEQ ID NO:116)

- The resulting PCR product was cloned in TOPO-XL (Invitrogen, Carlsbad, CA, USA), resulting in plasmid TOPO-XL-mev-op16. The insert carrying the mevalonate operon was excised with *Hind*III and *Sac*I and cloned in the *Hind*III-*Sac*I cut vector pBBR1MCS2 [Kovach et al., Gene 166:175-176 (1995)], resulting in plasmid pBBR-K-mev-op16.
- 5 Plasmid pBBR-K-mev-op16 was used to transform electroporation-competent *E. coli* strain TG1 [Stratagene, La Jolla, CA; Sambrook et al., In: Nolan, C. (ed.), Molecular Cloning: A Laboratory Manual (Second Edition), p. A.12 (1989)]. Two representative positive transformants (*E. coli* TG1/ pBBR-K-mev-op16-1 and *E. coli* TG1/ pBBR-K-mev-op16-2) were grown in Luria Broth (LB, GibcoBRL, Life Technologies) containing 50 mg/l
- 10 kanamycin and tested for HMG-CoA reductase activity (encoded by the *Paracoccus* sp. strain R114 *mvaA* gene) using the methods described in Example 1. *E. coli* does not possess a gene coding for the enzyme HMG-CoA reductase, hence the lack of detectable activity. The crude extracts of both representative transformants of *E. coli* TG1/ pBBR-K-mev-op16 had easily measurable HMG-CoA reductase activity, demonstrating the
- 15 heterologous expression of the cloned *mvaA* gene.

Table 17. HMG-CoA reductase activity in crude extracts of *E. coli* TG1 cells carrying the cloned mevalonate gene cluster from *Paracoccus* sp. strain R114.

Strain	HMG-CoA reductase activity (U/mg)
<i>E. coli</i> TG1	Not detected <sup>a</sup>
<i>E. coli</i> TG1/ pBBR-K-mev-op16-1	0.25
<i>E. coli</i> TG1/ pBBR-K-mev-op16-2	0.78

<sup>a</sup>Less than 0.03 U/mg

- Cloning and expression of the *idi* gene and the individual mevalonate pathway genes from
- 20 *Paracoccus* sp. strain R114 in *E. coli*. The coding regions of the mevalonate operon genes from *Paracoccus* sp. strain R114 were amplified by PCR using the primers shown in Table 18. The primers were designed such that the ATG start codons constituted the second half of an *Nde*I site (cleavage recognition site CATATG), and *Bam*HI sites (GGATCC) were introduced immediately after the stop codons. All PCR products were cloned in the
- 25 pCR<sup>®</sup>2.1-TOPO vector. The names of the resulting vectors are listed in Table 19. Except for the mevalonate kinase gene, all genes contained restriction sites for *Bam*HI, *Nde*I or *Eco*RI, which had to be eliminated in order to facilitate later cloning steps. The sites were eliminated by introducing silent mutations using the QuikChange<sup>™</sup> site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) and the oligonucleotides shown in Table 20.
- 30 The mutagenized coding regions were excised from the TOPO-plasmids with *Bam*HI and *Nde*I and ligated with the *Bam*HI-*Nde*I cleaved expression vectors pDS-His and pDS.

- These expression vectors were derived from pDSNdeHis, which is described in Example 2 of EP 821,063. The plasmid pDS-His was constructed from pDSNdeHis by deleting a 857 bp *NheI* and *XbaI* fragment carrying a silent chloramphenicol acetyltransferase gene. The plasmid pDS was constructed from pDS-His by replacing a small *EcoRI-BamHI* fragment with the annealed primers S/D-1 (5' AATTAAAGGAGGGTTTCATATGAATTCG) (SEQ ID NO:117) and S/D-2 (5' GATCCGAATTCATATGAAACCCCTCCTTT) (SEQ ID NO:118).

Table 18: Oligonucleotides for the cloning of the mevalonate operon genes.

Gene	Forward primer		Reverse primer	
	Name	Sequence (5'-3')	Name	Sequence (5'-3')
<i>mvaA</i>	MvaA-Nde	AAGGCCTCATATGATTTC CCATACCCCGGT (SEQ ID NO:119)	MvaA-Bam	CGGGATCCTCATCGCTCCAT CTCCATGT (SEQ ID NO:120)
<i>idi</i>	Idi-Nde	AAGGCCTCATATGACCGA CAGCAAGGATCA (SEQ ID NO:121)	Idi-Bam	CGGGATCCTCATTGACGGAT AAGCGAGG (SEQ ID NO:122)
<i>hsc</i>	Hcs-Nde	AAGGCCTCATATGAAAGT GCCTAAGATGA (SEQ ID NO:123)	Hcs-Bam	CGGGATCCTCAGGCCTGCCG GTCCGACAT (SEQ ID NO:124)
<i>mvk</i>	Mvk-Nde <sup>1</sup>	AAGGCCTCATATGAGCAC CGGCAGGCCTGAAGCA (SEQ ID NO:125)	Mvk-Bam <sup>2</sup>	CGGGATCCTCATCCCTGCCC CGGCAGCGGTT (SEQ ID NO:126)
<i>pmk</i>	Pmk-Nde	AAGGCCTCATATGGATCA GGTCATCCGCGC (SEQ ID NO:127)	Pmk-Bam	CGGGATCCTCAGTCATCGAA AACAAGTC (SEQ ID NO:128)
<i>mvd</i>	Mvd-Nde	AAGGCCTCATATGACTGA TGCCGTCGCCGA (SEQ ID NO:129)	Mvd-Bam	CGGGATCCTCAACGCCCTC GAACGGCG (SEQ ID NO:130)

<sup>1</sup>The second codon TCA was changed to AGC (silent mutation - both codons encode serine).

<sup>2</sup>The last codon GGC was changed to GGA (silent mutation - both codons encode glycine).

Table 19: Names of expression plasmids and construction intermediates.

Gene	PCR fragments in pCR*2.1-TOPO	After first mutagenesis	After 2 <sup>nd</sup> mutagenesis	Genes in pDS	Genes in pDS-His
<i>mvaA</i>	TOPO- <i>mvaA</i> -BB	TOPO- <i>mvaA</i> -B	TOPO- <i>mvaA</i>	pDS- <i>mvaA</i>	pDS-His- <i>mvaA</i>
<i>idi</i>	TOPO-ORFX-B	TOPO- <i>idi</i>	n/a	pDS- <i>idi</i>	pDS-His- <i>idi</i>
<i>hsc</i>	TOPO- <i>hcs</i> -EN	TOPO- <i>hcs</i> -N	TOPO- <i>hcs</i>	pDS- <i>hcs</i>	pDS-His- <i>hcs</i>
<i>mvk</i>	TOPO- <i>mvk</i>	n/a	n/a	pDS- <i>mvk</i>	pDS-His- <i>mvk</i>
<i>pmk</i>	TOPO- <i>pmk</i> -B	TOPO- <i>pmk</i>	n/a	Nd	pDS-His- <i>pmk</i>
<i>mvd</i>	TOPO- <i>mvd</i> -B	TOPO- <i>mvd</i>	n/a	pDS- <i>mvd</i>	pDS-His- <i>mvd</i>

n/a: not applicable; nd: not done

Table 20: Oligonucleotides for site-directed mutagenesis.

Gene/ Site	Forward primer		Reverse primer	
	Name	Sequence (5'-3')	Name	Sequence (5'-3')
<i>mvaA</i> / <i>Bam</i> HI-1	Mva-B1up	CCGGCATTCGGGCGGC ATCCAGGTCGCTG (SEQ ID NO:131)	Mva-B1down	CAGCGAGACCTGGATG CCGCCGAATGCCGG (SEQ ID NO:132)
<i>mvaA</i> / <i>Bam</i> HI-2	Mva-B2up	CGTCAGGGCTGGATT CTGTCGGAATACCCG (SEQ ID NO:133)	Mva-B2down	CGGGTATTCCGACAGA ATCCAGCCCTGCACG (SEQ ID NO:134)
<i>idi</i> / <i>Bam</i> HI	Idi-Bup2	GGGCTGCGCGCCGGCA TCCGGCATTTTCGACG (SEQ ID NO:135)	Idi-Bdown2	CGTCGAAATGCCGGAT GCCGGCGCGCAGCCC (SEQ ID NO:136)
<i>hcs</i> / <i>Eco</i> RI	Hcs-Eup	GGGTGCGACGGCGGAG TTCTTCGATGCGCGG (SEQ ID NO:137)	Hcs-Edown	CCGCGCATCGAAGAAC TCGCCCGTCGACCC (SEQ ID NO:138)
<i>hcs</i> / <i>Nde</i> I	Hcs-Nup-c	CAGCCCGTCACATAC GACGAATACGTTGCC (SEQ ID NO:139)	Hcs-Ndown- c	GGCAACGTATTCTGCG TATGTGACGGCGTG (SEQ ID NO:140)
<i>pmk</i> / <i>Bam</i> HI	Pmk-Bup	GAGGCTCGGGCTTGGC TCCTCGGCGGCGGTG (SEQ ID NO:141)	Pmk-Bdown	CACCGCGCCGAGGAG CCAAGCCGAGCCTC (SEQ ID NO:142)
<i>mvd</i> / <i>Bam</i> HI	Mvd-Bup	CGGCACGCTGCTGGAC CCGGCGACGCTTC (SEQ ID NO:143)	Mvd-Bdown	GAAGGCTGCTCCCGGG TCCAGCAGCGTGCCG (SEQ ID NO:144)

- 5 *E. coli* strain M15 [Villarejo and Zabin, J. Bacteriol. 120:466-474 (1974)] carrying the *lacI* (lac repressor)-containing plasmid pREP4 (EMBL/GenBank accession number A25856) was transformed with the ligation mixtures and recombinant cells were selected for by



growth on LB-Agar plates supplemented with 100 mg/L ampicillin and 25 mg/L kanamycin. Positive clones containing the correct mevalonate operon gene insert were verified by PCR.

- For expression of the inserted genes, each of the *E. coli* strains were grown overnight at 37°C in LB medium containing 25 mg/L kanamycin and 100 mg/L ampicillin. The next day, 25 ml of fresh medium was inoculated with 0.5 ml of the overnight cultures and the new cultures were grown at 37°C. When the OD<sub>600</sub> of the cultures reached 0.4, expression of the cloned genes was induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM, and the incubation of the cultures (with shaking) was continued for four hours, after which the cells were collected by centrifugation.

- Crude extract preparation, HMG-CoA reductase assays, and IPP isomerase assays were performed as described in Example 1. Tables 21 and 22 show the HMG-CoA reductase and IPP isomerase activities, respectively, in the recombinant *E. coli* strains. Upon IPTG induction, strains M15/pDS-*mvaA* and M15/pDS-*idi* contained high levels of the HMG-CoA reductase and IPP isomerase activity, respectively. This illustrates the ability to over-express the mevalonate pathway genes (and overproduce their cognate gene products in an active form) from *Paracoccus* sp. strain R114 in *E. coli*.

Table 21. Induction of HMG-CoA reductase activity in *E. coli* strains over-expressing the cloned *mvaA* gene from *Paracoccus* sp. strain R114.

Strain/plasmid	IPTG Induction	HMG-CoA reductase activity (U/mg)
M15/pDS- <i>mvaA</i>	-	8.34
M15/pDS- <i>mvaA</i>	+	90.0
M15/pDS-His- <i>mvaA</i>	-	1.74
M15/pDS-His- <i>mvaA</i>	+	2.95
M15/pDS- <i>mva</i> <sup>a</sup>	-	0.05

- <sup>a</sup>M15/pDS-*mvd* was included as a negative control

Table 22. Induction of IPP isomerase activity in *E. coli* strains over-expressing the cloned *idi* gene from *Paracoccus* sp. strain R114.

Strain/plasmid	IPTG Induction	IPP isomerase activity (U/mg)
M15/pDS- <i>idi</i>	-	Not detected <sup>b</sup>
M15/pDS- <i>idi</i>	+	22.0
M15/pDS-His- <i>idi</i>	-	Not detected
M15/pDS-His- <i>idi</i>	+	Not detected
M15/pDS- <i>mvd</i> <sup>a</sup>	-	Not detected

<sup>a</sup>M15/pDS-*mvd* was included as a negative control

<sup>b</sup><1 U/mg

- 5 The crude extracts used for the enzyme assays were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). For strains *E. coli* M15/pDS-*mvaA* and *E. coli* M15/pDS-His-*mvaA*, the presence or absence of a highly expressed protein of the expected molecular mass (36.3 kD) correlated with the HMG-CoA reductase activity measured in the extracts (Table 21). The absence of the His-tagged protein could be
- 10 explained by reduced expression at the level of transcription or translation by instability of the mRNA or the protein. The crude extracts of *E. coli* M15/pDS-*idi* and *E. coli* M15/pDS-His-*idi* both showed highly expressed proteins of the expected molecular masses of 37.3 kD and 39.0 kD, respectively. However, only the extract from *E. coli* M15/pDS-*idi* had increased IPP isomerase activity (Table 22), indicating that the histidine-tagged form of
- 15 the enzyme was not functional under these conditions.

By SDS-PAGE analysis of crude extracts of *E. coli* strains over-expressing the other four genes of the *Paracoccus* sp. strain R114 mevalonate operon (*hcs*, *pmk*, *mvk*, and *mvd*, refer to Table 19) high expression of the native form of the enzyme was not detected upon IPTG induction, although some expression cannot be ruled out. On the other hand, high

20 expression was observed with the His-tagged form of all four proteins.

#### Example 6: Improved Zeaxanthin Production in *Paracoccus* sp. strain R114 by Over-Expression of the *crtE* Gene

Construction of pBBR-K-Zea4, pBBR-K-Zea4-up and pBBR-K-Zea4-down and effects of these plasmids on zeaxanthin production in *Paracoccus* sp. strain R114. The carotenoid

25 (*crt*) gene cluster of *Paracoccus* sp. strain R1534 was excised from plasmid pZea-4 [Pasmontes et al., Gene 185:35-41 (1997)] as an 8.3 kb *Bam*HI - *Eco*RI fragment. This fragment containing the *crt* gene cluster was ligated into the *Bam*HI and *Eco*RI-cut vector pBBR1MCS-2 (GenBank accession #U23751) resulting in pBBR-K-Zea4. Plasmid pBBR-

- K-Zea4 was introduced into *Paracoccus* sp. strain R114 by conjugation to test for improved zeaxanthin production. The control strain R114 and two independent isolates of strain R114/pBBR-K-Zea4 were tested for zeaxanthin production in shake flask cultures (using medium 362F/2, see Example 11). The data in Table 23 show that both recombinant
- 5 strains carrying plasmid pBBR-K-Zea4 produced significantly higher levels of zeaxanthin than R114, and had higher specific rates of production (mg zeaxanthin/OD<sub>660</sub>). This suggested that one or more of the genes within the cloned insert in pBBR-K-Zea4 encoded an enzyme(s) that was limiting for zeaxanthin production in *Paracoccus* sp. strain R114.

Table 23. Zeaxanthin production by strains R114 and R114/pBBR-K-Zea4.

Strain	24 Hours		48 Hours		72 Hours	
	ZXN <sup>a</sup> (mg/l)	Spec. Form. <sup>b</sup>	ZXN (mg/l)	Spec. Form.	ZXN (mg/l)	Spec. Form.
R114	54.5	2.2	81.7	4.1	78.1	4.5
R114/pBBR-K-Zea4 (clone 4)	41.0	3.0	100.7	5.2	97.6	6.2
R114/pBBR-K-Zea4 (clone 5)	41.1	3.1	110.5	5.7	102.1	6.5

10 <sup>a</sup>Zeaxanthin

<sup>b</sup>Specific Formation (mg ZXN/l/OD<sub>660</sub>)

- To localize the positive effect, two plasmid derivatives were created that contained sub-cloned regions of the cloned insert present in pBBR-K-Zea4. The "upstream" region of the pBBR-K-Zea4 insert, comprising ORF 5 and the genes *atoB* and *crtE*, (Pasamontes et al.;
- 15 supra) is flanked by unique sites for the restriction enzymes *Xba*I and *Avr*II. Plasmid pBBR-K-Zea4-down was constructed by digesting pBBR-K-Zea4 with these two enzymes and deleting the "upstream" region. Analogously, plasmid pBBR-K-Zea4-up was constructed by deletion of the "downstream" region within the cloned insert in pBBR-K-Zea4, using the restriction enzymes *Eco*RV and *Stu*I. The two new plasmids were transferred to
- 20 *Paracoccus* sp. strain R114 by conjugation. Zeaxanthin production was compared (shake flask cultures, same conditions as described above) in strains R114 (host control), R114/pBBR-K (empty vector control), R114/pBBR-K-Zea4-down and R114/pBBR-K-Zea4-up (Table 24). The data clearly showed that the positive effect on zeaxanthin production was a result of the presence in multiple copies of the cloned segment containing
- 25 ORF5, *atoB* and *crtE*, i.e., the insert present in plasmid pBBR-K-Zea4-up. A series of deletion plasmids was constructed from pBBR-K-Zea4-up. By introducing each of these plasmids into strain R114 and testing for zeaxanthin production, it was determined that it

was over-expression of the *crtE* gene that was providing the improved zeaxanthin production in strains R114/pBBR-K-Zea4 and pBBR-K-Zea4-up. This result is consistent with the activity of GGPP synthase (encoded by *crtE*) being limiting for zeaxanthin production in *Paracoccus* sp. strain R114. Using the methods described in Example 1, crude extract of strain R114/pBBR-K-Zea4-up was found to have 2.6-fold higher GGPP synthase activity than R114. To prove this directly, a new plasmid allowing over-expression of only the *crtE* gene was constructed as described in the following two sections.

Table 24. Zeaxanthin production by strains carrying deletion derivatives of plasmid pBBR-K-Zea4.

Strain	24 Hours		48 Hours		72 Hours	
	ZXN <sup>a</sup> (mg/l)	Spec. Form. <sup>b</sup>	ZXN (mg/l)	Spec. Form.	ZXN (mg/l)	Spec. Form.
R114	35.0	1.2	75.7	4.1	73.9	4.4
R114/pBBR-K	32.0	1.5	59.3	3.1	63.3	3.9
R114/pBBR-K-Zea4-up	51.5	2.2	98.8	5.5	85.5	5.7
R114/pBBR-K-Zea4-down	41.6	1.8	63.4	3.3	66.4	3.9

<sup>a</sup> Zeaxanthin

<sup>b</sup> Specific Formation (mg ZXN/l/OD<sub>660</sub>)

Construction of the expression vectors pBBR-K-*PcrtE* and pBBR-tK-*PcrtE*. The vector pBBR1MCS-2 was cut with *Bst*XI and *Bsu*36I and the larger fragment was ligated with the annealed oligonucleotides MCS-2 up

(5' TCAGAATTCGGTACCATATGAAGCTTGGATCCGGGG 3') (SEQ ID NO:145) and MCS-2 down (5' GGATCCAAGCTTCATATGGTACCGAATTC 3') (SEQ ID NO:146), resulting in vector pBBR-K-Nde. The 270 bp region upstream of the *crtE* gene in the carotenoid gene cluster from *Paracoccus* sp. strain R114, which contains the putative *crtE* promoter (*PcrtE*) including the ribosome binding site and the *crtE* start codon (Pasamontes et al., supra) was amplified from *Paracoccus* sp. strain R114 DNA by PCR with primers crtE-up (5' GGAATTCGCTGCTGAACGCGATGGCG 3') (SEQ ID NO:147) and crtE-down (5' GGGGTACCATATGTGCCTTCGTTGCGTCAGTC 3') (SEQ ID NO:148). The PCR product was cut with *Eco*RI and *Nde*I and inserted into the *Eco*RI-*Nde*I cut backbone of pBBR-K-Nde, yielding plasmid pBBR-K-*PcrtE*. An *Nde*I site, which contains the ATG start codon of *crtE*, was included in primer crtE-down. Hence, any introduced coding region with the start codon embedded in a *Nde*I site should be expressed using the ribosomal binding site of *crtE*. The plasmid pBBR-K-*PcrtE* was cut with *Bam*HI and the annealed oligonucleotides pha-t-up

(5' GATCCGGCGTGTGCGCAATTTAATTGCGCACACGCCCCCTGCGTTTAAAC 3')  
(SEQ ID NO:149) and pha-t-down

(5' GATCGTTTAAACGCAGGGGCGTGTGCGCAATTAATTGCGCACACGCG 3')  
(SEQ ID NO:150) were inserted. The insertion was verified by sequencing, and the version

- 5 of the plasmid having the oligos inserted in the orientation that reconstitutes the *Bam*HI site closer to the *PcrE* promoter was named pBBR-tK-*PcrE*. The inserted sequence carries the putative transcriptional terminator found between the *Paracoccus* sp. strain R114 *phaA* and *phaB* genes (see Example 10) and should, therefore, ensure proper termination of the transcripts initiated from the *PcrE* promoter.
- 10 Construction of plasmid pBBR-K-*PcrE*-*crtE*-3. To construct a multi-copy plasmid for increased expression of the *crtE* gene in the *Paracoccus* sp. strain R114 host, the *crtE* gene was amplified from plasmid p59-2 (Pasamontes et al., supra) by PCR using the primers *crtE*-Nde (5' AAGGCTCATATGACGCCCAAGCAGCAATT 3') (SEQ ID NO:151) and *crtE*-Bam (5' CGGGATCCTAGGCGCTGCGGCGGATG 3') (SEQ ID NO:152). The
- 15 amplified fragment was cloned in the pCR<sup>®</sup>2.1-TOPO vector, resulting in plasmid TOPO-*crtE*. The *Nde*I-*Bam*HI fragment from TOPO-*crtE* was subcloned in *Nde*I-*Bam*HI-digested plasmid pBBR-K-*PcrE*, yielding pBBR-K-*PcrE*-*crtE*. Finally, pBBR-K-*PcrE*-*crtE*-3 was constructed by replacing the smaller *Bgl*II fragment from pBBR-K-*PcrE*-*crtE* with the smaller *Bgl*II fragment from pBBR-K-Zea4-up. Plasmid pBBR-K-*PcrE*-*crtE*-3
- 20 was transferred to *Paracoccus* sp. strain R114 by electroporation. Using the methods described in Example 1, GGPP synthase activity in crude extracts was found to be 2.9-fold higher in strain R114/pBBR-K-*PcrE*-*crtE*-3 than in strain R114. This degree of elevated activity was similar to that observed in R114/pBBR-K-Zea4-up. Table 25 shows the zeaxanthin production by strain R114/pBBR-K-*PcrE*-*crtE*-3 was essentially identical to
- 25 strain R114/pBBR-K-Zea4-up.

Table 25. Comparison of zeaxanthin production by strains R114/pBBR-K-*PcrtE*-*crtE*-3 and R114/pBBR-K-*Zea4*-up.

Strain	24 Hours		48 Hours		72 Hours	
	ZXN <sup>a</sup> (mg/l)	Spec. Form. <sup>b</sup>	ZXN (mg/l)	Spec. Form.	ZXN (mg/l)	Spec. Form.
R114	49.0	1.6	83.9	3.3	97.8	4.3
R114/pBBR-K	42.6	1.8	73.7	3.8	88.8	4.9
R114/pBBR-K- <i>PcrtE</i> - <i>crtE</i> -3	64.6	2.9	127.0	5.8	165.6	8.5
R114/pBBR-K- <i>Zea4</i> -up	64.7	2.9	118.0	5.9	158.0	10.1

<sup>a</sup>Zeaxanthin

<sup>b</sup>Specific Formation (mg ZXN/l/OD<sub>660</sub>)

- 5 Example 7: Expression of Individual Genes of the *Paracoccus* sp. strain R114 Mevalonate Operon in the Native Host, *Paracoccus* sp. strain R114

Expression of individual cloned genes of the *Paracoccus* sp. strain R114 mevalonate operon in the *Paracoccus* sp. strain R114 host. The mutagenized coding regions of the mevalonate operon genes in TOPO-plasmids (see Example 5) were excised with *Bam*HI and *Nde*I and  
 10 ligated with the *Bam*HI-*Nde*I cleaved vector pBBR-tK-*PcrtE* (see Example 6). The resulting plasmids pBBR-tK-*PcrtE*-*mvaA*, pBBR-tK-*PcrtE*-*idi*, pBBR-tK-*PcrtE*-*hcs*, pBBR-tK-*PcrtE*-*mvk*, pBBR-tK-*PcrtE*-*pmk* and pBBR-tK-*PcrtE*-*mvd* were introduced into *Paracoccus* sp. strain R114 by electroporation. Transformants were selected on agar medium containing 50 mg/l kanamycin and verified by PCR.

- 15 To illustrate that the plasmid-borne mevalonate pathway genes can be expressed in the native host *Paracoccus* sp. strain R114, HMG-CoA reductase activity was compared in crude extracts of strains R114/pBBR-K (control) and R114/pBBR-tK-*PcrtE*-*mvaA* (methods used are set forth in Example 1). The specific activities of HMG-CoA reductase in strains R114/pBBR-K and R114/pBBR-tK-*PcrtE*-*mvaA* were 2.37 U/mg and 6.0 U/mg,  
 20 respectively. Thus the presence of the *mvaA* gene on a multicopy plasmid (and expressed from the *PcrtE* promoter) resulted in a 2.5-fold increase in HMG-CoA reductase activity relative to the basal (i.e., chromosomally encoded) activity of R114 carrying the empty vector pBBR-K.

**Example 8: Construction of "Mini-Operons" for Simultaneous Over-Expression the Cloned Genes of the Mevalonate Pathway with the *Paracoccus* sp. strain R114 *crtE* Gene**

- Plasmid constructions. As was shown in Example 6, introduction of plasmid pBBR-K-  
 5 *PcrE-crtE*-3 into *Paracoccus* sp. strain R114 resulted in increased production of zeaxanthin, indicating that GGPP synthase activity was rate limiting for zeaxanthin biosynthesis in strain R114. Example 7 further showed that genes coding for the enzymes of the mevalonate pathway could be over-expressed in the native host *Paracoccus* sp. strain R114, and resulted in increased activity of the encoded enzyme. However, none of the  
 10 recombinant strains of *Paracoccus* sp. strain R114 that carried plasmids containing each individual gene of the mevalonate operon showed increased zeaxanthin production compared to strain R114. It is possible that the benefit of over-expression of the genes of the mevalonate operon in *Paracoccus* sp. strain R114 could be masked by the downstream "bottleneck" in the zeaxanthin pathway (GGPP synthase). Creation of plasmids that allow  
 15 simultaneous over-expression of each mevalonate pathway gene (or perhaps combinations of these genes) together with *crtE* could relieve all rate limitations in the overall zeaxanthin biosynthetic pathway, thereby improving zeaxanthin production. The next section describes the construction of "mini-operons" designed to allow co-over-expression of *crtE* and each of the genes coding for the five enzymes of the mevalonate pathway.
- 20 The *crtE*, *mvaA*, *idi* and *mvk* genes were excised from the respective TOPO-plasmids (described in Examples 5 and 6) with *Bam*HI and *Nde*I and ligated with *Bam*HI-*Nde*I-cleaved vector pOCV-1 (described in Example 12). The *crtE* gene does not have an adenine as the last nucleotide of the coding region, and in addition, has a TAG rather than a TGA stop codon and an unsuitable distance between the stop codon and the *Bam*HI site.  
 25 Therefore, the end of *crtE* does not meet the requirements of the operon construction vectors (refer to Example 12) and *crtE* must be the last gene in any operon constructed with pOCV-1-*crtE*. To meet the requirement for an adenine as the first nucleotide of the second codon and the last nucleotide of the last codon, mutations had to be introduced in three genes of the mevalonate operon. The second codon of *pmk*, GAT, encoding Asp, was  
 30 changed into AAT, encoding Asn. The last codon of *mvd* ends with a T and the last codons of *pmk* and *hcs* end with C. Changing these nucleotides to A results in silent mutations except for *pmk* where the last amino acid is changed from Asp to Glu. Oligonucleotides were designed to introduce the necessary changes by PCR. The sequences of the oligonucleotides and the templates used for those PCR reactions are  
 35 shown in Table 26. All PCR products were cloned in the pCR<sup>®</sup>2.1-TOPO vector, resulting in plasmids TOPO-*mva*<sup>OCV</sup>, TOPO-*pmk*<sup>OCV</sup> and TOPO-*hcs*<sup>OCV</sup>. The inserts were excised

with *Nde*I and *Bam*HI and ligated with the *Nde*I-*Bam*HI cut backbone of pOCV-2 (see Example 12). The final cloning steps to assemble each of the "mini-operons" were analogous, and are illustrated by the representative scheme for construction of pBBR-K-*PcrE-mvaA-crtE-3*.

- 5 Table 26: Oligonucleotides and templates used for PCR in the construction of plasmids TOPO-*mva*<sup>OCV</sup>, TOPO-*pmk*<sup>OCV</sup> and TOPO-*hcs*<sup>OCV</sup>.

Gene	Forward primer		Reverse primer		Template
	Name	Sequence (5'-3')	Name	Sequence (5'-3')	
<i>Hcs</i>	Hcs-Nde	AAGGCTCATATGAAA GTGCCTAAGATGA (SEQ ID NO:123)	Hcs-mut3	CCGGATCCTCATGCCC TGCCGGTCGACATAG (SEQ ID NO:153)	pBBR-tK- <i>PcrE-hcs</i>
<i>Pmk</i>	Pmk-mut5	GAAGGCACATATGAAT CAGGTATCCGCGC (SEQ ID NO:154)	Pmk-mut3	GCCGGATCCTCATTC ATCGAAAACAAGTCC (SEQ ID NO:155)	pBBR-tK- <i>PcrE-pmk</i>
<i>Mvd</i>	Mvd-Nde	AAGGCTCATATGACT GATGCCGTCCGCGA (SEQ ID NO:129)	Mvd-mut3	ACGCCGGATCCTCAT CGCCCTCGAACGGC (SEQ ID NO:156)	pBBR-tK- <i>PcrE-mvd</i>

Example 9: Cloning and Sequencing of the *ispA* Gene Encoding FPP Synthase from *Paracoccus* sp. strain R114

- 10 Because FPP synthase lies in the central pathway for zeaxanthin biosynthesis in *Paracoccus* sp. strain R114, increasing the activity of this enzyme by increasing the dosage of the *ispA* gene has the potential to improve zeaxanthin production. For this reason, the *ispA* gene from *Paracoccus* sp. strain R114 was cloned and sequenced as follows. The amino acid sequences of six bacterial FPP synthases were obtained from public databases. These
- 15 sequences have several highly conserved regions. Two such regions, and the oligonucleotides used for PCR, are shown in Table 27. PCR with oligonucleotides GTT-1 and GTT-2, using *Paracoccus* sp. strain R114 DNA as template, gave a product of the expected size. The PCR product was cloned in the vector pCR<sup>+</sup>2.1-TOPO and sequenced. The cloned
- 20 fragment was used as a probe for a Southern analysis of *Paracoccus* sp. strain R114 DNA and was found to hybridize to a *Bam*HI-*Nco*I fragment of about 1.9 kb. *Paracoccus* sp. strain R114 DNA was cut with *Bam*HI and *Nco*I and the fragments were separated by agarose gel electrophoresis. The region between 1.5 and 2.1 kb was isolated and cloned in the *Bam*HI and *Nco*I sites of a cloning vector. This partial library was then screened using the *ispA*-PCR fragment as a probe, and two positive clones were isolated. Sequencing con-
- 25 firmed that the plasmids of both clones contained the *ispA* gene. Upstream of *ispA* (SEQ ID NO:159) is the gene for the small subunit of exonuclease VII, XseB (SEQ ID NO:158),



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and downstream is the *dxs* gene (SEQ ID NO:160) encoding the 1-deoxyxylulose-5-phosphate synthase. This is the same gene arrangement as found in *E. coli*. The sequence of the *NcoI*-*BamHI* fragment is illustrated in SEQ ID NO:157, the amino acid sequences of XseB, IspA and Dxs are illustrated in SEQ ID NO:158, SEQ ID NO:159, and SEQ ID NO:160, respectively. The start codon of *ispA* may be GTG or ATG resulting in two or one methionine residues, respectively, at the amino-terminus of the native IspA.

Using the same general cloning strategy described in Examples 5-7, a new plasmid, pBBR-tk-*P<sub>prtE</sub>*-*ispA*-2 was constructed to allow for over-expression of the *ispA* gene in the native host *Paracoccus* sp. strain R114. The plasmid was introduced into strain R114 by electroporation, and transformants were confirmed by PCR. Three representative transformants and a control strain (R114/pBBR-K) were grown in 362F/2 medium (Example 11), crude extracts were prepared and assayed for activity of the *ispA* gene product, FPP synthase according to the methods described in Example 1. The basal (chromosomally-encoded) FPP synthase specific activity in R114/pBBR-K was 62.6 U/mg. The FPP synthase activity in the three transformants was 108.3 U/mg (73% increase), 98.5 U/mg (57% increase) and 83.8 U/mg (34% increase), demonstrating the over-expression of the *ispA* gene and over-production of its product, FPP synthase, in an active form in *Paracoccus* sp. strain R114.

Table 27: Oligonucleotides designed from two conserved bacterial IspA peptides.

Peptide 1	
<i>Bradyrhizobium japonicum</i>	Val His Asp Asp Leu Pro (SEQ ID NO:161)
<i>Rhizobium</i> sp. strain NGR234	Val His Asp Asp Leu Pro (SEQ ID NO:162)
<i>Bacillus stearothermophilus</i>	Ile His Asp Asp Leu Pro (SEQ ID NO:163)
<i>Bacillus subtilis</i>	Ile His Asp Asp Leu Pro (SEQ ID NO:164)
<i>Escherichia coli</i>	Ile His Asp Asp Leu Pro (SEQ ID NO:165)
<i>Haemophilus influenzae</i>	Ile His Asp Asp Leu Pro (SEQ ID NO:166)
Oligonucleotide GTT-1 (5'-3')	tc cay gay gay ctg cc (SEQ ID NO:167)
Peptide 2	
<i>Bradyrhizobium japonicum</i>	Asp Asp Ile Leu Asp (SEQ ID NO:168)
<i>Rhizobium</i> sp. strain NGR234	Asp Asp Ile Leu Asp (SEQ ID NO:169)
<i>Bacillus stearothermophilus</i>	Asp Asp Ile Leu Asp (SEQ ID NO:170)
<i>Bacillus subtilis</i>	Asp Asp Ile Leu Asp (SEQ ID NO:171)
<i>Escherichia coli</i>	Asp Asp Ile Leu Asp (SEQ ID NO:172)
<i>Haemophilus influenzae</i>	Asp Asp Ile Leu Asp (SEQ ID NO:173)
Reverse complement of	
Oligonucleotide GTT-2 (5'-3')	gay gay atc ctg gay (SEQ ID NO:174)

Y = C or T

**Example 10: Cloning and Sequencing of the Genes Coding for Acetyl-CoA  
Acetyltransferase from *Paracoccus* sp. strain R114**

The first committed step in IPP biosynthesis is the condensation of acetyl-CoA and aceto-  
 5 acetyl-CoA to hydroxymethylglutaryl-CoA (HMG-CoA) by HMG-CoA synthase. The sub-  
 strate acetoacetyl-CoA is formed by the enzyme acetyl-CoA acetyltransferase (also known  
 as acetoacetyl-CoA thiolase or  $\beta$ -ketothiolase) by condensation of two molecules of acetyl-  
 CoA. Because this reaction links central metabolism (at acetyl-CoA) to isoprenoid biosyn-  
 10 thesis via the mevalonate pathway, increasing the activity of acetyl-CoA acetyltransferase  
 by gene amplification has the potential to increase carbon flow to carotenoids and other  
 isoprenoids *in vivo*. In *Paracoccus* sp. strain R114, there are at least two genes, *atoB* and  
*phaA*, that encode acetyl-CoA acetyltransferases. The end of the *atoB* gene is 165 nucleo-  
 tides upstream of the start of *crtE* in *Paracoccus* sp. strains R1534 (US 6,087,152) and R114  
 (this work). The nucleotide sequence of the *atoB* gene and the corresponding amino acid  
 15 sequence of the encoded acetyl-CoA acetyltransferase from *Paracoccus* sp. strain R1534 are  
 illustrated in SEQ ID NO:175 and SEQ ID NO:176, respectively.

Using the same general strategy as described in Example 5, the *atoB* gene was cloned in  
 plasmids pDS and pDS-His. The new plasmids, pDS-*atoB* and pDS-His-*atoB* were intro-  
 duced into *E. coli* strain M15. The resulting strains M15/pDS-*atoB* and M15/pDS-His-  
 20 *atoB* were grown with and without IPTG induction (as described in Example 5), and crude  
 extracts were prepared for acetyl-CoA acetyltransferase assays (methods used were  
 described in Example 1) and SDS-PAGE analysis. The acetyl-CoA acetyltransferase  
 specific activities in extracts of M15/pDS-*atoB* and M15/pDS-His-*atoB* (with IPTG  
 induction) were 0.2 U/mg and 13.52 U/mg, respectively. The basal activity measured in *E.*  
 25 *coli* without the plasmids was 0.006 U/mg. Upon IPTG induction the *atoB* gene product,  
 acetyl-CoA acetyltransferase, is overproduced in *E. coli* M15. Both the native (M15/pDS-  
*atoB*) and His-tagged (M15/pDS-His-*atoB*) forms were overproduced. The degree of  
 overproduction was much higher in M15/pDS-His-*atoB*, consistent with the measured  
 acetyl-CoA acetyltransferase activity in the (induced) extracts of the two strains.

30 Acetoacetyl-CoA is also the substrate for the first committed step in poly-  
 hydroxyalkanoate (PHA) biosynthesis. In many bacteria the genes involved in PHA  
 biosynthesis are grouped in operons [Madison and Huisman, Microbiol. Mol. Biol. Rev.,  
 63:21-53 (1999)]. In *Paracoccus denitrificans* the *phaA* and *phaB* genes, encoding the  
 acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase, respectively, are clustered in

an operon [Yabutani et al., FEMS Microbiol. Lett. 133:85-90 (1995)] whereas *phaC*, the gene encoding the last enzyme in the pathway, poly(3-hydroxyalkanoate) synthase, is not part of this operon [Ueda et al., J. Bacteriol. 178:774-779 (1995)]. PCR fragments containing parts of *phaA* from *Paracoccus* sp. strain R1534 and *phaC* from *Paracoccus* sp. strain R114 were obtained using primers based on the *P. denitrificans* *phaA* and *phaC* gene sequences. The PCR fragments were then used as probes to screen a *Paracoccus* sp. strain R114  $\lambda$ -library (see Example 4). Several  $\lambda$ -clones hybridizing with the *phaA* or the *phaC* probes were isolated, and the presence of the *phaA* or *phaC* genes in the inserts was verified by sequence analysis. Three *phaA*  $\lambda$ -clones were further analyzed by subcloning and sequencing, whereby the *phaB* was found downstream of *phaA*. Therefore, as is the case in *P. denitrificans*, the *phaA* and *phaB* genes are clustered whereas the *phaC* gene is located elsewhere in the genome. The nucleotide sequence of the *phaAB* cluster from *Paracoccus* sp. strain R114 and the deduced amino acid sequences of the acetyl-CoA acetyltransferase (PhaA) are illustrated in SEQ ID NO:177, and SEQ ID NOs:178 and 179, respectively. The clustering of genes involved in PHA biosynthesis in operons suggests that at least *phaA* and *phaB* are expressed together when the cell produces poly(3-hydroxyalkanoates). On the other hand, a putative transcriptional stop signal is found between the *phaA* and *phaB* genes from *Paracoccus* sp. strain R114 which is absent in the *P. denitrificans* *phaAB* operon (Yabutani et al., supra). Thus, the expression of the two genes might not be coupled in *Paracoccus* sp. strain R114.

Using the same general strategy as described in Example 5, the *phaA* gene was cloned in plasmid pDS-His. The new plasmid, pDS-His-*phaA*, was introduced into *E. coli* strain M15. The resulting strain M15/pDS-His-*phaA* was grown with and without IPTG induction (as described in Example 5) and crude extracts were prepared for SDS-PAGE analysis. The cloned His-tagged *Paracoccus* sp. strain R114 PhaA (acetyl-CoA acetyltransferase) is overproduced upon IPTG induction in the *E. coli* M15 host.

The potential benefit of amplifying the *atoB* or *phaA* genes, encoding acetyl-CoA acetyltransferase, on zeaxanthin production is mentioned above. In addition, it may be beneficial for zeaxanthin production to decrease or eliminate the activity of acetoacetyl-CoA reductase (the *phaB* gene product) to avoid diversion of some of the acetoacetyl-CoA formed *in vivo* to the PHA pathway. Mutants of *Paracoccus* sp. strain R114 lacking activity of *phaB* could be obtained by gene replacement techniques (specifically replacing the wild-type *phaB* gene in the chromosome with an inactive form of the gene) or by classical mutagenesis and screening.

Example 11: Model for the Industrial Production of Lycopene Using Mutants Derived from *Paracoccus* sp. strain R114

- Lycopene is a red carotenoid that is an intermediate in the biosynthesis of zeaxanthin in the new *Paracoccus* species represented by strain R-1512 and its mutant derivatives R1534 and R114. As lycopene itself has significant commercial potential, it was of interest to test the potential of the new *Paracoccus* species to produce lycopene by industrial fermentation. To obtain mutants blocked in zeaxanthin biosynthesis that accumulated lycopene, *Paracoccus* sp. strain R114 was subjected to mutagenesis with ultraviolet (UV) light followed by screening for red colonies. The UV mutagenesis was performed as follows.
- 10 An overnight culture of strain R114 was grown in ME medium (see Example 2). The overnight culture was subcultured into fresh ME medium (initial  $OD_{610} = 0.1$ ) and incubated at 28°C for 3 hours. Aliquots of this culture were centrifuged and the pellet washed with 20mM potassium phosphate buffer (pH 7.2). After a second centrifugation, the pellet was resuspended to a final  $OD_{610}$  of 0.1. Ten milliliter aliquots of the cell
- 15 suspension were placed in a sterile 100-ml glass beaker. The thin layer of cell suspension was irradiated with UV light at a flux of  $1450\mu W/cm^2$  for a pre-determined optimal length of time. The cell suspension was mixed during the irradiation by means of a paper clip in the beaker and a magnetic stirrer. The mutagenized cell suspensions (and the unmutagenized controls) were plated on 362/F2 agar medium (Table 28). Triplicate viable
- 20 plate counts (in dim room light) were done on suspensions before and after mutagenesis. Plates were incubated for 4-5 days at 28°C, and the colonies were scored. Several red colonies (putative lycopene producers) were identified and purified by re-streaking. One mutant, designated UV7-1, was further evaluated for lycopene production.

- Table 29 shows the zeaxanthin production and lycopene production by the control strain R114 and its mutant derivative UV7-1. Strain R114 produced only zeaxanthin. Mutant UV7-1 produced mostly lycopene, but also produced a residual amount of zeaxanthin, suggesting that the mutational block in UV7-1 (presumably in the *crY* gene) is not complete. These results show that it is possible to derive lycopene producing strains from *Paracoccus* sp. strain R114.
- 25

Table 28. Recipe and preparation for medium 362F/2

Component	Amount
Glucose monohydrate	33 g
Yeast extract	10 g
Tryptone	10 g
NaCl	5 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.5 g
Agar (for solid medium)	20 g
Distilled water	To 932 ml
-adjust pH to 7.4	
-sterilize by filtration (liquid medium) or autoclaving (solid medium)	
-Add 2.5 ml each of microelements solution, NKP solution and CaFe solution	
Microelements solution	Amount per liter distilled water
(NH <sub>4</sub> ) <sub>2</sub> Fe(SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	80 g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	6 g
MnSO <sub>4</sub> ·H <sub>2</sub> O	2 g
NiSO <sub>4</sub> ·6H <sub>2</sub> O	0.2 g
EDTA	6 g
-sterilize by filtration	
NKP solution	Amount per liter distilled water
K <sub>2</sub> HPO <sub>4</sub>	250 g
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	300 g
-sterilize by filtration	
CaFe solution	Amount per liter distilled water
CaCl <sub>2</sub> ·2H <sub>2</sub> O	75 g
FeCl <sub>3</sub> ·6H <sub>2</sub> O	5 g
Concentrated HCl	3.75 ml
-sterilize by filtration	

Table 29. Zeaxanthin and lycopene production by *Paracoccus* sp. strain R114 and its red mutant derivative UV7-1.

	Zeaxanthin (mg/l)	Lycopene (mg/l)
24 hours		
R114	36.65	0
UV7-1	3.85	20.85
48 hours		
R114	72.95	0
UV7-1	5.75	85.95
72 hours		
R114	83.9	0
UV7-1	5.85	124.55

Example 12: Model for the Industrial Production of Astaxanthin by Fermentation Using

5

Strains Derived from *Paracoccus* sp. strain R114

Astaxanthin is a commercially important carotenoid used primarily in the aquaculture industry. EP 872,554 showed that astaxanthin production could be achieved in *E. coli* by introducing plasmids containing combinations of the cloned carotenoid (*crt*) genes from *Paracoccus* sp. strain R1534 and *Paracoccus carotinifaciens* E-396<sup>T</sup>. Together, the cloned *crt* genes (*crtE* and *crtW*) and *crtW* ( $\beta$ -carotene  $\beta$ -4 oxygenase) encoded a total biosynthetic pathway from FPP through zeaxanthin to astaxanthin. The sequences of the *P. carotinifaciens* E-396 *crtW*, *Paracoccus* sp. R1534 *crtZ*, and *Paracoccus* sp. R1534 *crtE* genes and encoded polypeptides are set forth in (SEQ ID NOs:180 and 181 (*crtW*); 182 and 184 (*crtZ*); and 184 and 185 (*crtE*)). However, it was not shown that astaxanthin production could be achieved in the *Paracoccus* sp. strain R114 host family. To demonstrate the utility of recombinant strains derived from strain R114 for astaxanthin production, the cloned *crtW* gene (SEQ ID NO:180) was introduced into strain R114 as follows.

10

15

Table 30. PCR primers used for the work described in Example 12.

Primer name	Sequence
CrtW-Nde	5' AAGGCCTCATATGAGCGCACATGCCCTGCC 3' (SEQ ID NO:186)
CrtW-Bam	5' CGGGATCCTCATGCGGTGCCCTTGG 3' (SEQ ID NO:187)
CrtZ-Nde	5' AAGGCCTCATATGAGCACTTGGGCCGAAT 3' (SEQ ID NO:188)
CrtZ-Bam	5' AGGATCCTCATGTATTGCGATCCGCCCTT 3' (SEQ ID NO:189)

The *crtW* gene was amplified by PCR from the cloned *crt* cluster of *Paracoccus carotini-faciens* strain E-396<sup>T</sup> (Tsubokura et al., supra; EP 872,554) using the primers *crtW*-Nde and *crtW*-Bam (Table 30). The primers were designed such that the ATG start codon constitutes the second half of an *NdeI* site (cleavage recognition site CATATG), and a *BamHI* site (GGATCC) was introduced immediately after the stop codon. The PCR product was  
5 cloned in the pCR<sup>®</sup>2.1-TOPO vector, resulting in plasmid TOPO-*crtW*. The *crtW* gene was excised with *NdeI* and *BamHI* and subcloned in the *NdeI*-*BamHI* cut vector pBBR-K-*PcrtE* (described in Example 6) to create plasmid pBBR-K-*PcrtE*-*crtW*.

Plasmid pBBR-K-*PcrtE*-*crtW* was transferred to *Paracoccus* sp. strain R114 using a standard  
10 bacterial conjugation procedure [*E. coli* strain S17 [Priefer et al., J. Bacteriol. 163:324-330 (1985)] was the donor organism]. Transconjugants were selected on medium 362F/2 agar (Table 28) containing 50 mg/l kanamycin and purified by restreaking on the same medium. The presence of plasmid pBBR-K-*PcrtE*-*crtW* in the strain was confirmed by  
15 PCR. Carotenoid production by strains R114 (host control), R114/pBBR-K (empty vector control) and R114/pBBR-K-*PcrtE*-*crtW* was measured in shake flask cultures as described in Examples 1 and 2, except that liquid 362F/2 medium was used instead of ME medium. These results are shown in Table 31. The control strains R114 and R114/pBBR-K produced only zeaxanthin. In strain R114/pBBR-K-*PcrtE*-*crtW*, the zeaxanthin was completely  
20 consumed by the plasmid-encoded  $\beta$ -carotene  $\beta$ -4 oxygenase. However, although astaxanthin was produced, two other ketocarotenoids, adonixanthin and canthaxanthin, accumulated at higher levels. This indicated an imbalance *in vivo* of the  $\beta$ -carotene hydroxylase (encoded by the chromosomal *crtZ* gene in strain R114) and the cloned  $\beta$  carotene  $\beta$ -4 oxygenase (*CrtW*).

To test this hypothesis, two new plasmids were created that contained the *crtZ* and *crtW* genes together in mini-operons. The order of the genes was made different in the two  
25 constructs (i.e., *crtZ*-*crtW* and *crtW*-*crtZ*) to try and create different ratios of expression of the *crtZ* and *crtW* genes. The construction of the new plasmids required the assembly of a special set of cloning vectors as follows. A series of operon construction vectors (based on the vector pCR<sup>®</sup>2.1-TOPO) was designed to facilitate the assembly of genes (in this case,  
30 *crtZ* and *crtW*) into operons. The genes of interest must have an ATG start codon, embedded in an *NdeI* site (CATATG), and a TGA stop codon immediately followed by a *BamHI* site.

Table 31. Astaxanthin production in *Paracoccus* sp. strain R114 containing plasmids expressing the *crtW* gene alone and in combination with the *crtZ* gene.

Strain	24 hours					
	ZXN	ADN	CXN	AXN	Total	Sp. Form. <sup>a</sup>
R114	46.5	0	0	0	46.5	2.1
R114/pBBR-K	38.8	0	0	0	41.4	2.2
R114/pBBR-K- <i>PcrtE</i> - <i>crtW</i>	0	13.0	21.8	2.3	37.5	2.1
R114/pBBR-K- <i>PcrtE</i> - <i>crtWZ</i>	0	14.9	29.5	1.3	45.6	2.1
R114/pBBR-K- <i>PcrtE</i> - <i>crtZW</i>	0	18.0	20.4	7.3	45.65	2.1
Strain	48 hours					
	ZXN	ADN	CXN	AXN	Total	Sp. Form. <sup>a</sup>
R114	72.6	0	0	0	74.4	2.8
R114/pBBR-K	70.1	0	0	0	70.1	3.1
R114/pBBR-K- <i>PcrtE</i> - <i>crtW</i>	0	26.7	22.0	26.9	75.5	3.9
R114/pBBR-K- <i>PcrtE</i> - <i>crtWZ</i>	0	30.9	27.2	34.8	92.9	4.0
R114/pBBR-K- <i>PcrtE</i> - <i>crtZW</i>	0	15.7	11.2	58.3	85.1	4.1
Strain	72 hours					
	ZXN	ADN	CXN	AXN	Total	Sp. Form. <sup>a</sup>
R114	82.5	0	0	0	82.5	5.3
R114/pBBR-K	82.9	0	0	0	82.9	5.1
R114/pBBR-K- <i>PcrtE</i> - <i>crtW</i>	0	19.7	17.0	46.8	83.5	5.2
R114/pBBR-K- <i>PcrtE</i> - <i>crtWZ</i>	0	28.7	26.4	43.8	98.8	6.1
R114/pBBR-K- <i>PcrtE</i> - <i>crtZW</i>	0	18.3	14.4	66.3	98.9	5.9

<sup>a</sup>ZXN, zeaxanthin; ADN, adonixanthin; CXN, canthaxanthin; AXN, astaxanthin.

<sup>b</sup>Specific Formation, expressed as mg/l total carotenoid/OD<sub>660</sub>.

- 5 Furthermore, the first nucleotide after the start codon and the last nucleotide before the stop codon must be adenine and the gene must lack sites for at least one of the enzymes *Bgl*I, *Bse*III, *Bse*RI and *Gsu*I. Four operon construction vectors were constructed, differing in the arrangements of their polylinker sequences (SEQ ID NOS: 190-197). The cleavage sites of the first two enzymes are within the *Nde*I site. The cleavage sites of the
- 10 last two enzymes are before the *Bam*HI site. The *Bse*RI site in pOCV-1 and pOCV-4 is not unique and cannot be used for operon construction.

The genes to be assembled in operons are first inserted individually between the *Nde*I and the *Bam*HI sites of the appropriate operon construction vectors. The resulting plasmid with the upstream gene of the envisioned operon is then cut with one of the two enzymes



at the end of the polylinker and with an enzyme, which has a unique site within the vector backbone. The plasmid containing the downstream gene of the envisioned operon is cut with one of the first two enzymes of the polylinker and with the same enzyme (with a unique site in the vector backbone) used for the first plasmid (containing the desired upstream gene). The fragments carrying the genes are isolated and ligated, resulting in a pOCV plasmid with both genes between the *NdeI* and the *BamHI* sites. More genes can be added in an analogous fashion. The assembled genes overlap such that the first two nucleotides, TG, of the TGA stop codon of the upstream gene coincide the last two nucleotides of the ATG start codon of the downstream gene. The same overlap is found between all genes in the carotenoid (*crt*) operon (*crtZYIB*) in *Paracoccus* sp. strain R1534 (Pasamontes et al., supra).

The pOCV backbone is derived from pCR<sup>\*</sup>2.1-TOPO. The *BseMII* site in the region necessary for replication, upstream of the *ColE1* origin, was eliminated by site directed mutagenesis changing the site from CTAG into CACAG. The remaining three *BseMII* sites and one *GsuI* site were eliminated by removing a 0.8 kb *DdeI*-*Asp700* fragment. The remaining vector was blunt-end ligated after fill-in of the *DdeI* recessed end. The poly-linkers were inserted between the *BamHI* and *XbaI* sites by means of annealed oligonucleotides with the appropriate 5' overhangs.

Plasmid pBBR-K-*PcrtE-crtZW*, was constructed using the operon construction vector pOCV-2 as follows. The *crtZ* gene was amplified by PCR from *Paracoccus* sp. strain R114 using the primers *crtZ*-*Nde* and *crtZ*-*Bam* (Table 30). The primers were designed such that the ATG start codon constitutes the second half of a *NdeI* site (cleavage recognition site CATATG) and a *BamHI* site (GGATCC) was introduced immediately after the stop codon. The PCR product was cloned in the pCR<sup>\*</sup>2.1-TOPO vector, resulting in plasmid TOPO-*crtZ*. To assemble the two genes in a mini-operon, both genes, *crtZ* and *crtW* were excised with *NdeI* and *BamHI* from the plasmids TOPO-*crtZ* and TOPO-*crtW* and sub-cloned in the *NdeI*-*BamHI* cut vector pOCV-2, creating plasmids pOCV-2-*crtZ* and pOCV-2-*crtW*. Plasmid pOCV-2-*crtZ* was cut with *BseMII* and *PstI* (there is a unique *PstI* site in the kanamycin resistance gene) and the 2.4 kb fragment (containing *crtZ*) was ligated with the *crtW*-containing 1876 bp *BseRI*-*PstI* fragment from pOCV-2-*crtW*. The resulting plasmid, pOCV-2-*crtZW*, was cut with *NdeI* and *BamHI* and the *crtZW* fragment was ligated with the *NdeI*-*BamHI* backbone of pBBR-K-*PcrtE* to yield pBBR-K-*PcrtE-crtZW*. Plasmid pBBR-K-*PcrtE-crtWZ*, was constructed in an analogous fashion.

The data in Table 31 show that the ratio of adonixanthin, canthaxanthin and astaxanthin did not change appreciably in strain R114/ pBBR-K-*PcrtE-crtWZ* compared to strain

- pBBR-K-*PrtE-crtW*. However, in strain pBBR-K-*PrtE-crtZW*, the production of the ketocarotenoids was shifted in favor of astaxanthin. This result indicates that the level of expression is dependent on the position of the gene within the mini-operon, and suggests that increasing the *in vivo* level of  $\beta$ -carotene hydroxylase activity creates a balance
- 5 between the activities of this enzyme and  $\beta$ -carotene  $\beta$ -4 oxygenase that is more favorable for full conversion of zeaxanthin to astaxanthin.

- The results described in this Example also show that it is possible, through appropriate genetic engineering, to produce not only astaxanthin, but also other ketocarotenoids of commercial interest in *Paracoccus* sp. strain R114 or its relatives. For example, expression
- 10 of a gene coding for  $\beta$ -carotene  $\beta$ -4 oxygenase in a *crtZ* mutant of strain R114 (lacking  $\beta$ -carotene hydroxylase activity) would provide for production of exclusively ketocarotenoids, e.g., echinenone or canthaxanthin, without co-production of hydroxylated carotenoids. Taken together, the results presented in this Example and Example 11 show the broad utility of *Paracoccus* sp. strain R114 and its relatives to produce industrially im-
- 15 portant carotenoids.

**Example 13: Accumulation of mevalonate in cultures of *Paracoccus* sp. strain R114 overexpressing genes of the mevalonate pathway**

- Overexpression of the genes of the mevalonate pathway in *Paracoccus* sp. strain R114 leads to increased carbon flow to through the mevalonate pathway. The construction of plasmid
- 20 pBBR-K-mev-op16-2 was described in Example 5. Plasmid pBBR-K-mev-op-up-4 was constructed as follows. A DNA fragment containing containing most of the *mvaA* gene and the entire *idi* and *hcs* genes was obtained on a 3.1 kb *SmaI-SalI* fragment following partial digestion of a  $\lambda$ -clone containing the *Paracoccus* sp. strain R114 mevalonate operon (see Example 4). This fragment was subcloned in pUC19, yielding the plasmid
- 25 pUC19mev-op-up'. To facilitate subcloning, the *KpnI-HindIII* fragment of pUC19mev-op-up' containing the mevalonate genes was recloned in the vector pBluescriptKS<sup>+</sup>, resulting in plasmid pBluKSp-mev-op-up'. A 1.7 kb *SalI* fragment from pUC19mev-op-up' was then cloned in the *SalI* site of plasmid 2ES2-1, which is a pUC19-derived plasmid containing the cloned *SalI-EcoRI* fragment M from *Paracoccus* sp. strain R114 (refer to
- 30 Example 4). This resulted in plasmid pUC19mev-op-up-2. Plasmid pUCmev-op-up-3 was then obtained by combining the *BbsI-BsaI* fragment from pUC19mev-op-up-2 carrying the beginning of the mevalonate operon with the *BbsI-BsaI* fragment from pBluKSp-mev-op-up' containing *idi* and *hcs*. Separately, a unique *MluI* site was introduced between the *NsiI* and *KpnI* sites of the vector pBBR1MCS-2 (refer to Example
- 35 5) by inserting an annealed primer containing an *MluI* restriction site. The resulting new

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cloning vector pBBR-K-Mlu was cut with *Mlu*I and *Kpn*I and the *Mlu*I-*Kpn*I fragment from pUCmev-op-up-3, containing the first three genes of the mevalonate operon, was inserted, yielding plasmid pBBR-K-mev-op-up-3. Plasmid pBBR-K-mev-op-up-4 was then constructed by insertion of the *Sma*I fragment from plasmid 16SB3, which contains most of the *mvk* gene and the 5' end of *pmk* (plasmid 16SB3 is a pUC19-derived plasmid containing the *Paracoccus* sp. strain R114 *Sa*II-*Bam*HI fragment A; refer to Example 4). The insert of plasmid pBBR-K-mev-op-up-4 contains the putative mevalonate operon promoter region, the first four genes of the mevalonate operon and the 5' end of *pmk*.

Plasmids pBBR-K-mev-op16-2 and pBBR-K-mev-op-up-4 were each introduced into *Paracoccus* sp. strain R114 by electroporation. Production of zeaxanthin and mevalonate by the new strains were compared to the control strain R114. The strains were grown in baffled shake flasks in liquid medium 362F/2 (see Example 11) for 72 hours. For strains R114/pBBR-K-mev-op16-2 and R114/pBBR-K-mev-op-up-4, kanamycin (50 mg/l) was also added to the cultures. The cultivation temperature was 28°C and shaking was at 200 rpm. Zeaxanthin was measured by the method set forth in Example 1, while mevalonate in the culture supernatants was measured as follows: A 0.6 ml sample of the culture was centrifuged for 4 minutes at 13,000 x g. Four hundred microliters of the supernatant were added to 400 microliters of methanol and mixed by vortexing for 1 min. The mixture was centrifuged again for 4 minutes at 13,000 x g. The resulting supernatant was then analyzed directly by gas chromatography (GC) using the method of Lindemann et al. [J. Pharm. Biomed. Anal. 9:311-316 (1991)] with minor modification as follows. The GC was a Hewlett-Packard 6890plus instrument (Hewlett-Packard, Avondale, PA, USA) equipped with a cool-on-column injector and a flame ionization detector. One microliter of sample prepared as described above was injected onto a fused silica capillary column (15m length x 0.32mm ID) coated with a 0.52 micron film of crosslinked modified polyethylene glycol (HP-FFAP, Agilent Technologies, USA). Helium was used as the carrier gas at an inlet pressure of 0.6 bar. The temperature of the programmable injector was ramped from 82°C to 250°C at a rate of 30°C/minute. The column temperature profile was 80°C for 0.5 minutes, followed by a linear temperature gradient at 15°C/min to 250°C and finally held at 250°C for 5 minutes. The detector temperature was maintained at 320°C.

In the first experiment, zeaxanthin and mevalonate production were measured in strains R114 and R114/pBBR-K-mev-op16-2 (Table 32). Both strains produced similar amounts of zeaxanthin, but strain R114/pBBR-K-mev-op16-2 produced a four-fold higher level of mevalonate. These results show that overexpression of the genes of the mevalonate pathway in *Paracoccus* sp. strain R114 results in increased carbon flow through the mevalonate

- pathway. The accumulation of mevalonate was expected because strain R114/pBBR-K-mev-op16-2 does not have an overexpressed *crfE* gene, and the *crfE* gene product (GGPP synthase) is known to be a limiting step in zeaxanthin production in *Paracoccus* sp. strain R114 (see Examples 6 and 8). Cells having a limiting amount of GGPP synthase, upon
- 15 overproduction of the enzymes of the mevalonate pathway, would be expected to accumulate FPP, and it is well known that FPP is a potent inhibitor of mevalonate kinase [Dorsey and Porter, J. Biol. Chem. 243:4667-4670 (1968); Gray and Kekwick, BBA 279:290-296 (1972); Hinson et al. J. Lipids Res. 38:2216-2223 (1997)]. Therefore, accumulation of
- 20 FPP resulting from overexpression of the genes of the mevalonate pathway would cause inhibition of mevalonate kinase, which in turn is manifested as mevalonate accumulation in the culture.

Table 32. Zeaxanthin and mevalonate production in strains R114 and R114/pBBR-K-mev-op16-2.

Strain/plasmid	Mevalonate (mg/l)	Zeaxanthin(mg/l)
R114	50.5	70.0
R114/pBBR-K-mev-op16-2	208.2	65.2

- 15 In a second experiment, zeaxanthin and mevalonate production were measured in strain R114 and two independent isolates of R114/pBBR-K-mev-op-up-4 (Table 33). These results again show that overexpression of the genes of the mevalonate pathway increased carbon flow through the mevalonate pathway.

Table 33. Zeaxanthin and mevalonate production in strains R114 and R114/pBBR-K-mev-op-up-4.

20

Strain/plasmid	Mevalonate (mg/l)	Zeaxanthin(mg/l)
R114	45	67.5
R114/pBBR-K-mev-op-up-4 (Isolate 1)	133.2	53.7
R114/pBBR-K-mev-op-up-4 (Isolate 2)	163.7	47.6

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The following biological material was deposited under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, VA 20110-2201, USA, and were assigned the following accession numbers:

Strain	Accession No.	Date of Deposit
<i>Paracoccus</i> sp. R114	PTA-3335	April 24, 2001
<i>Paracoccus</i> sp. R1534	PTA-3336	April 24, 2001
<i>Paracoccus</i> sp. R-1506	PTA-3431	June 5, 2001

- 5 All patents, patent applications, and publications cited above are incorporated herein by reference in their entirety as if recited in full herein.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of  
10 the following claims.

What is Claimed Is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (a) an amino acid sequence shown as residues 1 to 340 of SEQ ID NO:43;
  - 5 (b) an amino acid sequence shown as residues 1 to 349 of SEQ ID NO:45;
  - (c) an amino acid sequence shown as residues 1 to 388 of SEQ ID NO:47;
  - (d) an amino acid sequence shown as residues 1 to 378 of SEQ ID NO:49;
  - (e) an amino acid sequence shown as residues 1 to 305 of SEQ ID NO:51;
  - (f) an amino acid sequence shown as residues 1 to 332 of SEQ ID NO:53;
  - 10 (g) a fragment of an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, wherein said fragment has at least 30 contiguous amino acid residues;
  - (h) an amino acid sequence of a fragment of a polypeptide selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, the fragment having the activity of
  - 15 hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase), mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase;
  - (i) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive
  - 20 nucleotides of SEQ ID NO:42 or a complement of SEQ ID NO:42, wherein the polypeptide has the activity of HMG-CoA reductase, isopentenyl diphosphate isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; and
  - (j) a conservatively modified variant of SEQ ID NO:43, 45, 47, 49, 51 or 53.
- 25 2. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (a) an amino acid sequence shown as residues 1 to 287 of SEQ ID NO:159;
  - (b) at least 30 contiguous amino acid residues of SEQ ID NO:159;
  - (c) an amino acid sequence of a fragment of SEQ ID NO: 159, the fragment having the
  - 30 activity of farnesyl diphosphate synthase (FPP synthase);
  - (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 295-1158 of SEQ ID NO:157 or a complement thereof, wherein the polypeptide has the activity of FPP synthase; and
  - 35 (e) a conservatively modified variant of SEQ ID NO:159.

3. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
- (a) an amino acid sequence shown as residues 1 to 142 of SEQ ID NO:160;
  - (b) at least 30 contiguous amino acid residues of SEQ ID NO:160;
  - 5 (c) an amino acid sequence of a fragment of SEQ ID NO: 160, the fragment having the activity of 1-deoxyxylulose-5-phosphate synthase (DXPS);
  - (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1185-1610 of SEQ ID NO:157 or a complement thereof,
  - 10 wherein the polypeptide has the activity of DXPS;
  - (e) a conservatively modified variant of SEQ ID NO:160.
4. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
- (a) an amino acid sequence shown as residues 1 to 390 of SEQ ID NO:178;
  - 15 (b) at least 30 contiguous amino acid residues of SEQ ID NO:178;
  - (c) an amino acid sequence of a fragment of a polypeptide of SEQ ID NO: 178, the fragment having the activity of acetyl-CoA acetyltransferase;
  - (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive
  - 20 nucleotides spanning positions 1-1170 of SEQ ID NO:177 or a complement thereof,
  - wherein the polypeptide has the activity of acetyl-CoA acetyltransferase; and
  - (e) a conservatively modified variant of SEQ ID NO:178.
5. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
- 25 (a) an amino acid sequence shown as residues 1 to 240 of SEQ ID NO:179;
  - (b) at least 30 contiguous amino acid residues of SEQ ID NO:179;
  - (c) an amino acid sequence of a fragment of a polypeptide of SEQ ID NO: 179, the fragment having the activity of acetoacetyl-CoA reductase;
  - (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes
  - 30 under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1258-1980 of SEQ ID NO:177 or a complement thereof,
  - wherein the polypeptide has the activity of acetoacetyl-CoA reductase; and
  - (e) a conservatively modified variant of SEQ ID NO:179.
6. An isolated polynucleotide sequence comprising a nucleotide sequence selected from the
- 35 group consisting of SEQ ID NO:42, variants of SEQ ID NO:42 containing one or more

substitutions according to the *Paracoccus* sp. strain R1534 codon usage table, fragments of SEQ ID NO:42 that encode a polypeptide having an activity selected from the group consisting of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase),

5 mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:42, or the complement of SEQ ID NO:42, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of HMG-CoA reductase,

10 isopentenyl diphosphate isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase.

7. An isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:157, variants of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain

15 R1534 codon usage table, fragments of SEQ ID NO:157 that encode a polypeptide having farnesyl diphosphate (FPP) synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity or a polypeptide having the activity of XseB, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:157, or the complement of

20 SEQ ID NO:157, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of FPP synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity, and the activity of XseB.

8. An isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:177, variants of SEQ

25 ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table, fragments of SEQ ID NO:177 that encode a polypeptide having an activity selected from the group consisting of acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30

30 contiguous nucleotides of SEQ ID NO:177, or the complement of SEQ ID NO:177, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase.

9. An isolated polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:42, SEQ ID NO:157, SEQ ID NO:177, and combinations

35 thereof.



10. An expression vector comprising the polynucleotide sequence according to claim 6, 7, 8 or 9.
11. An expression vector selected from the group consisting of pBBR-K-mev-op16-1, pBBR-K-mev-op16-2, pDS-*mvaA*, pDS-*idi*, pDS-*hcs*, pDS-*mvk*, pDS-*pmk*, pDS-*mvd*, pDS-*His-mvaA*, pDS-*His-idi*, pDS-*His-hcs*, pDS-*His-mvk*, pDS-*His-pmk*, pDS-*His-mvd*, pBBR-K-*Zea4*, pBBR-K-*Zea4-up*, pBBR-K-*Zea4-down*, pBBR-K-*PcrtE-crtE-3*, pBBR-tK-*PcrtE-mvaA*, pBBR-tK-*PcrtE-idi*, pBBR-tK-*PcrtE-hcs*, pBBR-tK-*PcrtE-mvk*, pBBR-tK-*PcrtE-pmk*, pBBR-tK-*PcrtE-mvd*, pBBR-K-*PcrtE-mvaA-crtE-3*, pDS-*His-phaA*, pBBR-K-*PcrtE-crtW*, pBBR-K-*PcrtE-crtWZ*, pBBR-K-*PcrtE-crtZW*, and combinations thereof.
12. A cultured cell comprising the polynucleotide sequence according to claim 6, 7, 8 or 9, or an expression vector according to claim 10 or 11, or a progeny of the cell, wherein the cell expresses a polypeptide encoded by the polynucleotide sequence.
13. A method of producing a carotenoid comprising culturing a cell according to claim 12 under conditions permitting expression of a polypeptide encoded by the polynucleotide sequence, and isolating the carotenoid from the cell or the medium of the cell.
14. A method of making a carotenoid-producing cell comprising:
- (a) introducing into a cell a polynucleotide sequence encoding an enzyme in the mevalonate pathway, which enzyme is expressed in the cell; and
  - (b) selecting a cell containing the polynucleotide sequence of step (a) that produces a carotenoid at a level that is about 1.1-1,000 times the level of the carotenoid produced by the cell before introduction of the polynucleotide sequence.
15. A method for engineering a bacterium to produce an isoprenoid compound comprising:
- (a) culturing a parent bacterium in a medium under conditions permitting expression of an isoprenoid compound, and selecting a mutant bacterium from the culture medium that produces about 1.1-1,000 times more of an isoprenoid compound than the parent bacterium;
  - (b) introducing into the mutant bacterium an expression vector comprising a polynucleotide sequence represented by SEQ ID NO:42 operably linked to an expression control sequence; and
  - (c) selecting a bacterium that contains the expression vector and produces at least about 1.1 times more of an isoprenoid compound than the mutant in step (a).

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16. A microorganism of the genus *Paracoccus*, which microorganism has the following characteristics:

- (i) a sequence similarity to SEQ ID NO:12 of >97% using a similarity matrix obtained from a homology calculation using GeneCompar v. 2.0 software with a gap penalty of 0%;
- 5 a homology to strain R-1512, R1534, R114 or R-1506 of >70% using DNA:DNA hybridization at 81.5°C;
- a G+C content of its genomic DNA that varies less than 1% from the G+C content of the genomic DNA of R114, R-1512, R1534, and R-1506; and
- an average DNA fingerprint that clusters at about 58% similarity to strains R-1512, R1534,
- 10 R114 and R-1506 using the AFLP procedure of Example 2, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966);
- (ii) 18:1w7c comprising at least about 75% of the total fatty acids of the cell membranes;
- an inability to use adonitol, i-erythritol, gentiobiose,  $\beta$ -methylglucoside, D-sorbitol, xylitol and quinic acid as carbon sources for growth; and
- 15 an ability to use L-asparagine and L-aspartic acid as carbon sources for growth, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966); or
- (iii) an ability to grow at 40°C;
- an ability to grow in a medium having 8% NaCl;
- an ability to grow in a medium having a pH of 9.1; and
- 20 a yellow-orange colony pigmentation, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966).

## SEQUENCE LISTING

&lt;110&gt; BERRY, Alan

BRETZEL, Werner

HUMBELIN, Markus

LOPEZ-ULIBARRI, Rual

MAYER, Anne

YELISEEV, Alexei

&lt;120&gt; IMPROVED ISOPRENOID PRODUCTION

&lt;130&gt; C38435/121966

&lt;160&gt; 197

&lt;170&gt; PatentIn version 3.0

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&lt;212&gt; PRT

&lt;213&gt; Paracoccus sp. R114

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Arg Val Arg Phe Cys Glu Arg Leu Lys His Gly Lys Ala Gly Pro Ile
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<213> Paracoccus sp. R114

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Ile Asp Pro Arg Leu Gly Leu Gly Ser Ser Ala Ala Val Thr Val Ala  
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Cys Leu Gly Ala Leu Ser Arg Leu Ala Gly Arg Gly Thr Glu Gly Leu  
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Gly Ala Asp Leu Ala Ala Ser Leu His Gly Gly Phe Val Ala Tyr Arg  
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Gly Pro Phe Gly Leu Arg Tyr Ala Gly Tyr Lys Thr Pro Thr Ala Glu  
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Val Leu Arg Leu Val Ala Asp Arg Met Ala Gly Asn Glu Ala Ala Phe  
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Asp Ala Leu Tyr Ser Arg Met Gly Ala Ser Ala Asp Ala Ala Ile Arg  
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Asp  
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&lt;211&gt; 9066

&lt;212&gt; DNA

<213> *Paracoccus* sp. R114

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&lt;223&gt; mvd

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&lt;211&gt; 332

&lt;212&gt; PRT

&lt;213&gt; Paracoccus sp. R114

&lt;400&gt; 53

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 Ser Lys Tyr Trp Gly Lys Arg Asp Ala Ala Arg Asn Leu Pro Leu Asn  
 35 40 45



Ser Ser Val Ser Ile Ser Leu Ala Asn Trp Gly Ser His Thr Arg Val  
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Glu Gly Ser Gly Thr Gly His Asp Glu Val His His Asn Gly Thr Leu  
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Leu Asp Pro Gly Asp Ala Phe Ala Arg Arg Ala Leu Ala Phe Ala Asp  
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Leu Phe Arg Gly Gly Arg His Leu Pro Leu Arg Ile Thr Thr Gln Asn  
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Ser Ile Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Gly Phe Ala  
 115 120 125

Ala Leu Thr Arg Ala Leu Ala Gly Ala Phe Gly Leu Asp Leu Asp Asp  
 130 135 140

Thr Asp Leu Ser Arg Ile Ala Arg Ile Gly Ser Gly Ser Ala Ala Arg  
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Ser Ile Trp His Gly Phe Val Arg Trp Asn Arg Gly Glu Ala Glu Asp  
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Gly His Asp Ser His Gly Val Pro Leu Asp Leu Arg Trp Pro Gly Phe  
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Arg Ile Ala Ile Val Ala Val Asp Lys Gly Pro Lys Pro Phe Ser Ser  
 195 200 205

Arg Asp Gly Met Asn His Thr Val Glu Thr Ser Pro Leu Phe Pro Pro  
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Trp Pro Ala Gln Ala Glu Ala Asp Cys Arg Val Ile Glu Asp Ala Ile  
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Ala Ala Arg Asp Met Ala Ala Leu Gly Pro Arg Val Glu Ala Asn Ala  
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Leu Ala Met His Ala Thr Met Met Ala Ala Arg Pro Pro Leu Cys Tyr  
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Leu Thr Gly Gly Ser Trp Gln Val Leu Glu Arg Leu Trp Gln Ala Arg  
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Ala Asp Gly Leu Ala Ala Phe Ala Thr Met Asp Ala Gly Pro Asn Val  
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Pro Asp Ala Ser Leu Ile Ala Pro Phe Glu Gly Arg  
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<211> 353

<212> PRT

<213> Streptomyces sp. strain CL190

<400> 54

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Lys Val Ser Arg Leu Thr Glu Lys Gly Ile Val Ala Thr Leu Val Asp  
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Glu Arg Met Thr Arg Ser Val Ile Val Glu Ala Thr Asp Ala Gln Thr  
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Ala Tyr Met Ala Ala Gln Thr Ile His Ala Arg Ile Asp Glu Leu Arg  
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Glu Val Val Arg Gly Cys Ser Arg Phe Ala Gln Leu Ile Asn Ile Lys  
 100 105 110

His Glu Ile Asn Ala Asn Leu Leu Phe Ile Arg Phe Glu Phe Thr Thr  
 115 120 125

Gly Asp Ala Ser Gly His Asn Met Ala Thr Leu Ala Ser Asp Val Leu  
 130 135 140

Leu Gly His Leu Leu Glu Thr Ile Pro Gly Ile Ser Tyr Gly Ser Ile

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    165                      170                      175  
 Leu Gly Arg Gly Lys Asn Val Ile Thr Glu Leu Leu Val Pro Arg Asp  
    180                      185                      190  
 Val Val Glu Asn Asn Leu His Thr Thr Ala Ala Lys Ile Val Glu Leu  
    195                      200                      205  
 Asn Ile Arg Lys Asn Leu Leu Gly Thr Leu Leu Ala Gly Gly Ile Arg  
    210                      215                      220  
 Ser Ala Asn Ala His Phe Ala Asn Met Leu Leu Gly Phe Tyr Leu Ala  
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    245                      250                      255  
 Met Ala Glu Asp Arg Asp Gly Asp Leu Tyr Phe Ala Cys Thr Leu Pro  
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 Asn Leu Ile Val Gly Thr Val Gly Asn Gly Lys Gly Leu Gly Phe Val  
    275                      280                      285  
 Glu Thr Asn Leu Ala Arg Leu Gly Cys Arg Ala Asp Arg Glu Pro Gly  
    290                      295                      300  
 Glu Asn Ala Arg Arg Leu Ala Val Ile Ala Ala Thr Val Leu Cys  
    305                      310                      315                      320  
 Gly Glu Leu Ser Leu Leu Ala Ala Gln Thr Asn Pro Gly Glu Leu Met  
    325                      330                      335  
 Arg Ala His Val Gln Leu Glu Arg Asp Asn Lys Thr Ala Lys Val Gly  
    340                      345                      350

Ala

&lt;210&gt; 55

&lt;211&gt; 353

&lt;212&gt; PRT

&lt;213&gt; Streptomyces griseolosporeus

&lt;400&gt; 55

Met Thr Glu Ala His Ala Thr Ala Gly Val Pro Met Arg Trp Val Gly  
 1                      5                      10                      15  
 Pro Val Arg Ile Ser Gly Asn Val Ala Thr Ile Glu Thr Gln Val Pro

20										25					30				
Leu	Ala	Thr	Tyr	Glu	Ser	Pro	Leu	Trp	Pro	Ser	Val	Gly	Arg	Gly	Ala				
35						40					45								
Lys	Val	Ser	Arg	Leu	Thr	Glu	Lys	Gly	Ile	Val	Ala	Thr	Leu	Val	Asp				
50						55					60								
Glu	Arg	Met	Thr	Arg	Ser	Val	Leu	Val	Glu	Ala	Thr	Asp	Ala	Leu	Thr				
65						70					75				80				
Ala	Leu	Ser	Ala	Ala	Arg	Thr	Ile	Glu	Ala	Arg	Ile	Asp	Glu	Leu	Arg				
85						90					95								
Glu	Leu	Val	Arg	Gly	Cys	Ser	Arg	Phe	Ala	Gln	Leu	Ile	Gly	Ile	Arg				
100						105					110								
His	Glu	Ile	Thr	Gly	Asn	Leu	Leu	Phe	Val	Arg	Phe	Glu	Phe	Ser	Thr				
115						120					125								
Gly	Asp	Ala	Ser	Gly	His	Asn	Met	Ala	Thr	Leu	Ala	Ser	Asp	Val	Leu				
130						135					140								
Leu	Gln	His	Leu	Leu	Glu	Thr	Val	Pro	Gly	Ile	Ser	Tyr	Gly	Ser	Ile				
145						150					155				160				
Ser	Gly	Asn	Tyr	Cys	Thr	Asp	Lys	Lys	Ala	Thr	Ala	Ile	Asn	Gly	Ile				
165						170					175								
Leu	Gly	Arg	Gly	Lys	Asn	Val	Val	Thr	Glu	Leu	Leu	Val	Pro	Arg	Asp				
180						185					190								
Val	Val	Ala	Asp	Val	Leu	Asn	Thr	Thr	Ala	Ala	Lys	Ile	Ala	Glu	Leu				
195						200					205								
Asn	Leu	Arg	Lys	Asn	Leu	Leu	Gly	Thr	Leu	Leu	Ala	Gly	Gly	Ile	Arg				
210						215					220								
Ser	Ala	Asn	Ala	His	Tyr	Ala	Asn	Met	Leu	Leu	Ala	Phe	Tyr	Leu	Ala				
225						230					235				240				
Thr	Gly	Gln	Asp	Ala	Ala	Asn	Ile	Val	Glu	Gly	Ser	Gln	Gly	Val	Val				
245						250					255								
Thr	Ala	Glu	Asp	Arg	Asp	Gly	Asp	Leu	Tyr	Leu	Ala	Cys	Thr	Leu	Pro				
260						265					270								
Asn	Leu	Ile	Val	Gly	Thr	Val	Gly	Asn	Gly	Lys	Gly	Leu	Gly	Phe	Val				
275						280					285								
Glu	Thr	Asn	Leu	Asn	Arg	Leu	Gly	Cys	Arg	Ala	Asp	Arg	Glu	Pro	Gly				
290						295					300								
Glu	Asn	Ala	Arg	Arg	Leu	Ala	Val	Ile	Ala	Ala	Ala	Thr	Val	Leu	Cys				
305						310					315				320				
Gly	Glu	Leu	Ser	Leu	Leu	Ala	Ala	Gln	Thr	Asn	Pro	Gly	Glu	Leu	Met				

[illegible]

195				200				205							
Asn	Ile	Arg	Lys	Asn	Leu	Leu	Gly	Thr	Leu	Leu	Ala	Gly	Gly	Ile	Arg
210							215					220			
Ser	Ala	Asn	Ala	His	Tyr	Ala	Asn	Met	Leu	Leu	Gly	Phe	Tyr	Leu	Ala
225						230					235				240
Thr	Gly	Gln	Asp	Ala	Ala	Asn	Ile	Val	Glu	Gly	Ser	Gln	Gly	Val	Thr
						245					250				255
Leu	Ala	Glu	Asp	Arg	Asp	Gly	Asp	Leu	Tyr	Phe	Ser	Cys	Asn	Leu	Pro
			260							265				270	
Asn	Leu	Ile	Val	Gly	Thr	Val	Gly	Asn	Gly	Lys	Gly	Leu	Glu	Phe	Val
			275				280							285	
Glu	Thr	Asn	Leu	Asn	Arg	Leu	Gly	Cys	Arg	Glu	Asp	Arg	Ala	Pro	Gly
			290				295							300	
Glu	Asn	Ala	Arg	Arg	Leu	Ala	Val	Ile	Ala	Ala	Ala	Thr	Val	Leu	Cys
305						310					315				320
Gly	Glu	Leu	Ser	Leu	Leu	Ala	Ala	Gln	Thr	Asn	Pro	Gly	Glu	Leu	Met
						325				330				335	
Arg	Ala	His	Val	Glu	Leu	Glu	Arg	Asp	Asn	Thr	Thr	Ala	Glu	Val	Gly
			340							345				350	

Val

&lt;210&gt; 57

&lt;211&gt; 347

&lt;212&gt; PRT

<213> *Erwinia herbicola*

&lt;400&gt; 57

Met	Lys	Asp	Glu	Arg	Leu	Val	Gln	Arg	Lys	Asn	Asp	His	Leu	Asp	Ile
1					5				10					15	
Val	Leu	Asp	Pro	Arg	Arg	Ala	Val	Thr	Gln	Ala	Ser	Ala	Gly	Phe	Glu
			20					25						30	
Arg	Trp	Arg	Phe	Thr	His	Cys	Ala	Leu	Pro	Glu	Leu	Asn	Phe	Ser	Asp
		35					40						45		
Ile	Thr	Leu	Glu	Thr	Thr	Phe	Leu	Asn	Arg	Gln	Leu	Gln	Ala	Pro	Leu
		50				55					60				
Leu	Ile	Ser	Ser	Met	Thr	Gly	Gly	Val	Glu	Arg	Ser	Arg	His	Ile	Asn

65		70		75		80
Arg His Leu Ala Glu	Ala Ala Gln Val Leu Lys Ile Ala Met Gly Val					
	85			90		95
Gly Ser Gln Arg Val Ala Ile Glu Ser Asp Ala Gly Leu Gly Leu Asp						
	100		105			110
Lys Thr Leu Arg Gln Leu Ala Pro Asp Val Pro Leu Leu Ala Asn Leu						
	115		120			125
Gly Ala Ala Gln Leu Thr Gly Arg Lys Gly Ile Asp Tyr Ala Arg Arg						
	130		135			140
Ala Val Glu Met Ile Glu Ala Asp Ala Leu Ile Val His Leu Asn Pro						
	145		150			155
Leu Gln Glu Ala Leu Gln Pro Gly Gly Asp Arg Asp Trp Arg Gly Arg						
	165		170			175
Leu Ala Ala Ile Glu Thr Leu Val Arg Glu Leu Pro Val Pro Leu Val						
	180		185			190
Val Lys Glu Val Gly Ala Gly Ile Ser Arg Thr Val Ala Gly Gln Leu						
	195		200			205
Ile Asp Ala Gly Val Thr Val Ile Asp Val Ala Gly Ala Gly Gly Thr						
	210		215			220
Ser Trp Ala Ala Val Glu Gly Glu Arg Ala Ala Thr Glu Gln Gln Arg						
	225		230			235
Ser Val Ala Asn Val Phe Ala Asp Trp Gly Ile Pro Thr Ala Glu Ala						
	245		250			255
Leu Val Asp Ile Ala Glu Ala Trp Pro Gln Met Pro Leu Ile Ala Ser						
	260		265			270
Gly Gly Ile Lys Asn Gly Val Asp Ala Ala Lys Ala Leu Arg Leu Gly						
	275		280			285
Ala Cys Met Val Gly Gln Ala Ala Ala Val Leu Gly Ser Ala Gly Val						
	290		295			300
Ser Thr Glu Lys Val Ile Asp His Phe Asn Val Ile Ile Glu Gln Leu						
	305		310			315
Arg Val Ala Cys Phe Cys Thr Gly Ser Arg Ser Leu Ser Asp Leu Lys						
	325		330			335
Gln Ala Asp Ile Arg Tyr Val Arg Asp Thr Pro						
	340		345			

<210> 58

<211> 360

&lt;212&gt; PRT

<213> *Borrelia burgdorferi*

&lt;400&gt; 58

Met Met Asp Thr Glu Phe Met Gly Ile Glu Pro Asn Ile Leu Glu Asn  
 1 5 10 15

Lys Lys Arg His Ile Glu Ile Cys Leu Asn Lys Asn Asp Val Lys Gly  
 20 25 30

Gly Cys Asn Phe Leu Lys Phe Ile Lys Leu Lys His Asn Ala Leu Ser  
 35 40 45

Asp Phe Asn Phe Ser Glu Ile Asn Ile Lys Glu Glu Ile Phe Gly Tyr  
 50 55 60

Asn Ile Ser Met Pro Val Phe Ile Ser Ser Met Thr Gly Gly Ser Lys  
 65 70 75 80

Glu Gly Asn Asp Phe Asn Lys Ser Leu Val Arg Ile Ala Asn Tyr Leu  
 85 90 95

Lys Ile Pro Ile Gly Leu Gly Ser Phe Lys Leu Leu Phe Lys Tyr Pro  
 100 105 110

Glu Tyr Ile Arg Asp Phe Thr Leu Lys Arg Tyr Ala His Asn Ile Pro  
 115 120 125

Leu Phe Ala Asn Val Gly Ala Val Gln Ile Val Glu Phe Gly Ile Ser  
 130 135 140

Lys Ile Ala Glu Met Ile Lys Arg Leu Glu Val Asp Ala Ile Ile Val  
 145 150 155 160

His Leu Asn Ala Gly Gln Glu Leu Met Lys Val Asp Gly Asp Arg Asn  
 165 170 175

Phe Lys Gly Ile Arg Glu Ser Ile Ala Lys Leu Ser Asp Phe Leu Ser  
 180 185 190

Val Pro Leu Ile Val Lys Glu Thr Gly Phe Gly Ile Ser Pro Lys Asp  
 195 200 205

Val Lys Glu Leu Phe Ser Leu Gly Ala Ser Tyr Val Asp Leu Ala Gly  
 210 215 220

Ser Gly Gly Thr Asn Trp Ile Leu Val Glu Gly Met Lys Ser Asn Asn  
 225 230 235 240

Leu Asn Ile Ala Ser Cys Phe Ser Asp Trp Gly Ile Pro Ser Val Phe  
 245 250 255

Thr Leu Leu Ser Ile Asp Asp Ser Leu Lys Ala Asn Ile Phe Ala Ser



260										265										270									
Gly	Gly	Tyr	Glu	Thr	Gly	Met	Asp	Ile	Ala	Lys	Gly	Ile	Ala	Leu	Gly														
275										280										285									
Ala	Arg	Leu	Ile	Gly	Val	Ala	Ala	Val	Val	Leu	Arg	Ala	Phe	Tyr	Asp														
290										295										300									
Ser	Gly	Glu	Asp	Ala	Val	Phe	Gly	Leu	Phe	Ser	Asp	Tyr	Glu	His	Ile														
305										310										315									
Leu	Lys	Met	Ser	Met	Phe	Leu	Ser	Gly	Ser	Lys	Ser	Leu	Leu	Glu	Phe														
320										325										330									
Arg	Asn	Asn	Lys	Tyr	Phe	Leu	Ser	Ser	Tyr	Leu	Leu	Asp	Glu	Leu	Gly														
335										340										345									
Val	Phe	Lys	Gln	Phe	Tyr	Gly	Thr																						
350										355										360									
<210>																59													
<211>																349													
<212>																PRT													
<213>																Synechocystis sp. PCC 6803													
<400>																59													
Met	Asp	Ser	Thr	Pro	His	Arg	Lys	Ser	Asp	His	Ile	Arg	Ile	Val	Leu														
1										5										15									
Glu	Glu	Asp	Val	Val	Gly	Lys	Gly	Ile	Ser	Thr	Gly	Phe	Glu	Arg	Leu														
20										25										30									
Met	Leu	Glu	His	Cys	Ala	Leu	Pro	Ala	Val	Asp	Leu	Asp	Ala	Val	Asp														
35										40										45									
Leu	Gly	Leu	Thr	Leu	Trp	Gly	Lys	Ser	Leu	Thr	Tyr	Pro	Trp	Leu	Ile														
50										55										60									
Ser	Ser	Met	Thr	Gly	Gly	Thr	Pro	Glu	Ala	Lys	Gln	Ile	Asn	Leu	Phe														
65										70										75									
Leu	Ala	Glu	Val	Ala	Gln	Ala	Leu	Gly	Ile	Ala	Met	Gly	Leu	Gly	Ser														
85										90										95									
Gln	Arg	Ala	Ala	Ile	Glu	Asn	Pro	Asp	Leu	Ala	Phe	Thr	Tyr	Gln	Val														
100										105										110									
Arg	Ser	Val	Ala	Pro	Asp	Ile	Leu	Leu	Phe	Ala	Asn	Leu	Gly	Leu	Val														
115										120										125									
Gln	Leu	Asn	Tyr	Gly	Tyr	Gly	Leu	Glu	Gln	Ala	Gln	Arg	Ala	Val	Asp														

130					135					140					
Met	Ile	Glu	Ala	Asp	Ala	Leu	Ile	Leu	His	Leu	Asn	Pro	Leu	Gln	Glu
145					150					155					160
Ala	Val	Gln	Pro	Asp	Gly	Asp	Arg	Leu	Trp	Ser	Gly	Leu	Trp	Ser	Lys
				165					170					175	
Leu	Glu	Ala	Leu	Val	Glu	Ala	Leu	Glu	Val	Pro	Val	Ile	Val	Lys	Glu
			180					185					190		
Val	Gly	Asn	Gly	Ile	Ser	Gly	Pro	Val	Ala	Lys	Arg	Leu	Gln	Glu	Cys
			195				200					205			
Gly	Val	Gly	Ala	Ile	Asp	Val	Ala	Gly	Ala	Gly	Gly	Thr	Ser	Trp	Ser
	210						215					220			
Glu	Val	Glu	Ala	His	Arg	Gln	Thr	Asp	Arg	Gln	Ala	Lys	Glu	Val	Ala
				230						235					240
His	Asn	Phe	Ala	Asp	Trp	Gly	Leu	Pro	Thr	Ala	Trp	Ser	Leu	Gln	Gln
				245					250					255	
Val	Val	Gln	Asn	Thr	Glu	Gln	Ile	Leu	Val	Phe	Ala	Ser	Gly	Gly	Ile
			260					265					270		
Arg	Ser	Gly	Ile	Asp	Gly	Ala	Lys	Ala	Ile	Ala	Leu	Gly	Ala	Thr	Leu
			275				280					285			
Val	Gly	Ser	Ala	Ala	Pro	Val	Leu	Ala	Glu	Ala	Lys	Ile	Asn	Ala	Gln
			290				295				300				
Arg	Val	Tyr	Asp	His	Tyr	Gln	Ala	Arg	Leu	Arg	Glu	Leu	Gln	Ile	Ala
				310					315					320	
Ala	Phe	Cys	Cys	Asp	Ala	Ala	Asn	Leu	Thr	Gln	Leu	Ala	Gln	Val	Pro
				325					330					335	
Leu	Trp	Asp	Arg	Gln	Ser	Gly	Gln	Arg	Leu	Thr	Lys	Pro			
			340				345								

<210> 60

<211> 361

<212> PRT

<213> Streptomyces sp. CL190

<400> 60

Met Thr Ser Ala Gln Arg Lys Asp Asp His Val Arg Leu Ala Ile Glu  
1 5 10 15  
Gln His Asn Ala His Ser Gly Arg Asn Gln Asp Asp Val Ser Phe Val

20					25					30					
His	His	Ala	Leu	Ala	Gly	Ile	Asp	Arg	Pro	Asp	Val	Ser	Leu	Ala	Thr
		35					40					45			
Ser	Phe	Ala	Gly	Ile	Ser	Trp	Gln	Val	Pro	Ile	Tyr	Ile	Asn	Ala	Met
	50					55					60				
Thr	Gly	Gly	Ser	Glu	Lys	Thr	Gly	Leu	Ile	Asn	Arg	Asp	Leu	Ala	Thr
65					70					75					80
Ala	Ala	Arg	Glu	Thr	Gly	Val	Pro	Ile	Ala	Ser	Gly	Ser	Met	Asn	Ala
			85						90					95	
Tyr	Ile	Lys	Asp	Pro	Cys	Ala	Asp	Thr	Phe	Arg	Val	Leu	Arg	Asp	Glu
			100					105					110		
Asn	Pro	Asn	Gly	Phe	Val	Ile	Ala	Asn	Ile	Asn	Ala	Thr	Thr	Thr	Val
		115					120					125			
Asp	Asn	Ala	Gln	Arg	Ala	Ile	Asp	Leu	Ile	Glu	Ala	Asn	Ala	Leu	Gln
	130					135					140				
Ile	His	Ile	Asn	Thr	Ala	Gln	Glu	Thr	Pro	Met	Pro	Glu	Gly	Asp	Arg
145					150					155					160
Ser	Phe	Ala	Ser	Trp	Val	Pro	Gln	Ile	Glu	Lys	Ile	Ala	Ala	Ala	Val
			165						170					175	
Asp	Ile	Pro	Val	Ile	Val	Lys	Glu	Val	Gly	Asn	Gly	Leu	Ser	Arg	Gln
			180					185					190		
Thr	Ile	Leu	Leu	Leu	Ala	Asp	Leu	Gly	Val	Gln	Ala	Ala	Asp	Val	Ser
		195					200					205			
Gly	Arg	Gly	Gly	Thr	Asp	Phe	Ala	Arg	Ile	Glu	Asn	Gly	Arg	Arg	Glu
	210					215						220			
Leu	Gly	Asp	Tyr	Ala	Phe	Leu	His	Gly	Trp	Gly	Gln	Ser	Thr	Ala	Ala
225					230					235					240
Cys	Leu	Leu	Asp	Ala	Gln	Asp	Ile	Ser	Leu	Pro	Val	Leu	Ala	Ser	Gly
			245						250					255	
Gly	Val	Arg	His	Pro	Leu	Asp	Val	Val	Arg	Ala	Leu	Ala	Leu	Gly	Ala
			260					265					270		
Arg	Ala	Val	Gly	Ser	Ser	Ala	Gly	Phe	Leu	Arg	Thr	Leu	Met	Asp	Asp
	275						280					285			
Gly	Val	Asp	Ala	Leu	Ile	Thr	Lys	Leu	Thr	Thr	Trp	Leu	Asp	Gln	Leu
	290					295					300				
Ala	Ala	Leu	Gln	Thr	Met	Leu	Gly	Ala	Arg	Thr	Pro	Ala	Asp	Leu	Thr
305					310					315					320
Arg	Cys	Asp	Val	Leu	Leu	His	Gly	Glu	Leu	Arg	Asp	Phe	Cys	Ala	Asp

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Arg Gly Ile Asp Thr Arg Arg Leu Ala Gln Arg Ser Ser Ser Ile Glu  
              340                        345                    350

Ala Leu Gln Thr Thr Gly Ser Thr Arg  
              355                        360

<210> 61

<211> 364

<212> PRT

<213> Streptomyces griseolosporus

<400> 61

Met Ser Ser Ala Gln Arg Lys Asp Asp His Val Arg Leu Ala Thr Glu  
1              5                        10                    15

Gln Gln Arg Ala His Ser Gly Arg Asn Gln Phe Asp Asp Val Ser Phe  
              20                        25                    30

Val His His Ala Leu Ala Gly Ile Asp Arg Pro Asp Val Arg Leu Ala  
              35                        40                    45

Thr Thr Phe Ala Gly Ile Thr Trp Arg Leu Pro Leu Tyr Ile Asn Ala  
50                        55                    60

Met Thr Gly Gly Ser Ala Lys Thr Gly Ala Ile Asn Arg Asp Leu Ala  
65                        70                    75                    80

Val Ala Ala Arg Glu Thr Gly Ala Ala Ile Ala Ser Gly Ser Met His  
              85                        90                    95

Ala Phe Phe Arg Asp Pro Ser Cys Ala Asp Thr Phe Arg Val Leu Arg  
              100                        105                    110

Thr Glu Asn Pro Asp Gly Phe Val Met Ala Asn Val Asn Ala Thr Ala  
              115                        120                    125

Ser Val Asp Asn Ala Arg Arg Ala Val Asp Leu Ile Glu Ala Asn Ala  
130                        135                    140

Leu Gln Ile His Leu Asn Thr Ala Gln Glu Thr Pro Met Pro Glu Gly  
145                        150                    155                    160

Asp Arg Ser Phe Gly Ser Trp Pro Ala Gln Ile Ala Lys Ile Thr Ala  
              165                        170                    175

Ala Val Asp Val Pro Val Ile Val Lys Glu Val Gly Asn Gly Leu Ser  
              180                        185                    190

Arg Gln Thr Leu Leu Ala Leu Pro Asp Leu Gly Val Arg Val Ala Asp

195                      200                      205  
 Val Ser Gly Arg Gly Gly Thr Asp Phe Ala Arg Ile Glu Asn Ser Arg  
 210                      215                      220  
 Arg Pro Leu Gly Asp Tyr Ala Phe Leu His Gly Trp Gly Gln Ser Thr  
 225                      230                      235                      240  
 Pro Ala Cys Leu Leu Asp Ala Gln Asp Val Gly Phe Pro Leu Leu Ala  
 245                      250                      255  
 Ser Gly Gly Ile Arg Asn Pro Leu Asp Val Ala Arg Ala Leu Ala Leu  
 260                      265                      270  
 Gly Ala Gly Ala Val Gly Ser Ser Gly Val Phe Leu Arg Thr Leu Ile  
 275                      280                      285  
 Asp Gly Gly Val Ser Ala Leu Val Ala Gln Ile Ser Thr Trp Leu Asp  
 290                      295                      300  
 Gln Leu Ala Ala Leu Gln Thr Met Leu Gly Ala Arg Thr Pro Ala Asp  
 305                      310                      315                      320  
 Leu Thr Arg Cys Asp Val Leu Ile His Gly Pro Leu Arg Ser Phe Cys  
 325                      330                      335  
 Thr Asp Arg Gly Ile Asp Ile Gly Arg Phe Ala Arg Arg Ser Ser Ser  
 340                      345                      350  
 Ala Asp Ile Arg Ser Glu Met Thr Gly Ser Thr Arg  
 355                      360  
 <210> 62  
 <211> 368  
 <212> PRT  
 <213> *Sulfolobus solfataricus*  
  
 <400> 62  
 Met Pro Asp Ile Val Asn Arg Lys Val Glu His Val Glu Ile Ala Ala  
 1                      5                      10                      15  
 Phe Glu Asn Val Asp Gly Leu Ser Ser Ser Thr Phe Leu Asn Asp Val  
 20                      25  
 Ile Leu Val His Gln Gly Phe Pro Gly Ile Ser Phe Ser Glu Ile Asn  
 35                      40                      45  
 Thr Lys Thr Lys Phe Phe Arg Lys Glu Ile Ser Ala Pro Ile Met Val  
 50                      55                      60  
 Thr Gly Met Thr Gly Gly Arg Asn Glu Leu Gly Arg Ile Asn Arg Ile



&lt;211&gt; 342

&lt;212&gt; PRT

&lt;213&gt; Rickettsia prowazekii

&lt;400&gt; 63

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Met Pro Lys Glu Gln Asn Leu Asp Ile Glu Arg Lys Gln Glu His Ile
 1           5           10           15

Glu Ile Asn Leu Lys Gln Asn Val Asn Ser Thr Leu Lys Ser Gly Leu
 20           25           30

Glu Ser Ile Lys Phe Ile His Asn Ala Leu Pro Glu Ile Asn Tyr Asp
 35           40           45

Ser Ile Asp Thr Thr Thr Thr Phe Leu Gly Lys Asp Met Lys Ala Pro
 50           55           60

Ile Leu Ile Ser Ser Met Thr Gly Gly Thr Ala Arg Ala Arg Asp Ile
 65           70           75           80

Asn Tyr Arg Leu Ala Gln Ala Ala Gln Lys Ser Gly Ile Ala Met Gly
 85           90           95

Leu Gly Ser Met Arg Ile Leu Leu Thr Lys Pro Asp Thr Ile Lys Thr
100           105           110

Phe Thr Val Arg His Val Ala Pro Asp Ile Pro Leu Leu Ala Asn Ile
115           120           125

Gly Ala Val Gln Leu Asn Tyr Gly Val Thr Pro Lys Glu Cys Gln Tyr
130           135           140

Leu Ile Asp Thr Ile Lys Ala Asp Ala Leu Ile Leu His Leu Asn Val
145           150           155           160

Leu His Glu Leu Thr Gln Pro Glu Gly Asn Lys Asn Trp Glu Asn Leu
165           170           175

Leu Pro Lys Ile Lys Glu Val Ile Asn Tyr Leu Ser Val Pro Val Ile
180           185           190

Val Lys Glu Val Gly Tyr Gly Leu Ser Lys Gln Val Ala Lys Lys Leu
195           200           205

Ile Lys Ala Gly Val Lys Val Leu Asp Ile Ala Gly Ser Gly Gly Thr
210           215           220

Ser Trp Ser Gln Val Glu Ala Tyr Arg Ala Lys Asn Ser Met Gln Asn
225           230           235           240

Arg Ile Ala Ser Ser Phe Ile Asn Trp Gly Ile Thr Thr Leu Asp Ser

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**245**
**250**
**255**

Leu Lys Met Leu Gln Glu Ile Ser Lys Asp Ile Thr Ile Ile Ala Ser  
260
265
270

Gly Gly Leu Gln Ser Gly Ile Asp Gly Ala Lys Ala Ile Arg Met Gly  
275
280
285

Ala Asn Ile Phe Gly Leu Ala Gly Lys Leu Leu Lys Ala Ala Asp Ile  
290
295
300

Ala Glu Ser Leu Val Leu Glu Glu Ile Gln Val Ile Ile Glu Gln Leu  
305
310
315
320

Lys Ile Thr Met Leu Cys Thr Gly Ser Cys Thr Leu Lys Asp Leu Ala  
325
330
335

Lys Ala Glu Ile Met Trp  
340

<210> 64

<211> 286

<212> PRT

<213> Deinococcus radiodurans

<400> 64

Met	Arg	Leu	Asp	Thr	Val	Phe	Leu	Gly	Arg	Arg	Leu	Lys	Ala	Pro	Val
1			5					10						15	
Leu	Ile	Gly	Ala	Met	Thr	Gly	Gly	Ala	Glu	Lys	Ala	Gly	Val	Ile	Asn
		20						25					30		
Arg	Asn	Leu	Ala	Thr	Ala	Ala	Arg	Asn	Leu	Gly	Leu	Gly	Met	Met	Leu
		35					40					45			
Gly	Ser	Gln	Arg	Val	Met	Leu	Glu	His	Pro	Asp	Ala	Trp	Glu	Ser	Phe
	50					55					60				
Asn	Val	Arg	Glu	Val	Ala	Pro	Glu	Ile	Leu	Leu	Ile	Gly	Asn	Leu	Gly
65					70					75					80
Ala	Ala	Gln	Phe	Met	Leu	Gly	Tyr	Gly	Ala	Glu	Gln	Ala	Arg	Arg	Ala
			85					90						95	
Val	Asp	Glu	Val	Met	Ala	Asp	Ala	Leu	Ala	Ile	His	Leu	Asn	Pro	Leu
		100						105					110		
Gln	Glu	Ala	Leu	Gln	Arg	Gly	Gly	Asp	Thr	Arg	Trp	Gln	Gly	Val	Thr
		115				120						125			
Tyr	Arg	Leu	Lys	Gln	Val	Ala	Arg	Glu	Leu	Asp	Phe	Pro	Val	Ile	Ile



130                      135                      140  
 Lys Glu Val Gly His Gly Leu Asp Ala Ala Thr Leu Arg Ala Leu Ala  
 145                      150                      155                      160  
 Asp Gly Pro Phe Ala Ala Tyr Asp Val Ala Gly Ala Gly Gly Thr Ser  
                     165                      170                      175  
 Trp Ala Arg Val Glu Gln Leu Val Ala His Gly Gln Val His Ser Pro  
                     180                      185                      190  
 Asp Leu Cys Glu Leu Gly Val Pro Thr Ala Gln Ala Leu Arg Gln Ala  
                     195                      200                      205  
 Arg Lys Thr Leu Pro Gly Ala Gln Leu Ile Ala Ser Gly Gly Ile Arg  
 210                      215                      220  
 Ser Gly Leu Asp Ala Ala Arg Ala Leu Ser Leu Gly Ala Glu Val Val  
 225                      230                      235                      240  
 Ala Val Ala Arg Pro Leu Leu Glu Pro Ala Leu Asp Ser Ser Glu Ala  
                     245                      250                      255  
 Ala Glu Ala Trp Leu Arg Asn Phe Ile Gln Glu Leu Arg Val Ala Leu  
                     260                      265                      270  
 Phe Val Gly Gly Tyr Arg Asp Val Arg Glu Val Arg Gly Gly  
                     275                      280                      285  
 <210> 65  
 <211> 361  
 <212> PRT  
 <213> Aeropyrum pernix  
  
 <400> 65  
 Met Ile Val Ser Ser Lys Val Glu Ser Arg Glu Ser Thr Leu Leu Glu  
 1                      5                      10                      15  
 Tyr Val Arg Ile Val His Asn Pro Thr Pro Glu Val Asn Leu Gly Asp  
                     20                      25                      30  
 Val Ser Leu Glu Ile Asp Phe Cys Gly Gly Arg Leu Arg Ala Pro Leu  
                     35                      40                      45  
 Val Ile Thr Gly Met Thr Gly Gly His Pro Asp Val Glu Trp Ile Asn  
                     50                      55                      60  
 Arg Glu Leu Ala Ser Val Ala Glu Glu Leu Gly Ile Ala Ile Gly Val  
 65                      70                      75                      80  
 Gly Ser Gln Arg Ala Ala Ile Glu Asp Pro Ser Leu Ala Arg Thr Phe

85										90										95																			
Arg	Ala	Ala	Arg	Glu	Ala	Ala	Pro	Asn	Ala	Phe	Leu	Ile	Ala	Asn	Leu					Arg	Ala	Ala	Arg	Glu	Ala	Ala	Pro	Asn	Ala	Phe	Leu	Ile	Ala	Asn	Leu				
			100						105																														
Gly	Ala	Pro	Gln	Leu	Ser	Leu	Gly	Tyr	Ser	Val	Arg	Glu	Val	Arg	Met					Gly	Ala	Pro	Gln	Leu	Ser	Leu	Gly	Tyr	Ser	Val	Arg	Glu	Val	Arg	Met				
			115					120																															
Ala	Val	Glu	Met	Ile	Asp	Ala	Asp	Ala	Ile	Ala	Ile	His	Leu	Asn	Pro					Ala	Val	Glu	Met	Ile	Asp	Ala	Asp	Ala	Ile	Ala	Ile	His	Leu	Asn	Pro				
			130				135																																
Gly	Gln	Glu	Ala	Tyr	Gln	Pro	Glu	Gly	Asp	Pro	Phe	Tyr	Arg	Gly	Val					Gly	Gln	Glu	Ala	Tyr	Gln	Pro	Glu	Gly	Asp	Pro	Phe	Tyr	Arg	Gly	Val				
					150					155																													
Val	Gly	Lys	Ile	Ala	Glu	Ala	Ala	Glu	Ala	Ala	Gly	Val	Pro	Val	Ile					Val	Gly	Lys	Ile	Ala	Glu	Ala	Ala	Glu	Ala	Gly	Val	Pro	Val	Ile					
			165						170																														
Val	Lys	Glu	Thr	Gly	Asn	Gly	Leu	Ser	Arg	Glu	Ala	Val	Ala	Gln	Leu					Val	Lys	Glu	Thr	Gly	Asn	Gly	Leu	Ser	Arg	Glu	Ala	Val	Ala	Gln	Leu				
			180					185																															
Arg	Ala	Leu	Gly	Val	Arg	Cys	Phe	Asp	Val	Ala	Gly	Leu	Gly	Gly	Thr					Arg	Ala	Leu	Gly	Val	Arg	Cys	Phe	Asp	Val	Ala	Gly	Leu	Gly	Gly	Thr				
			195				200																																
Asn	Trp	Ile	Lys	Ile	Glu	Val	Leu	Arg	Gly	Arg	Lys	Ala	Gly	Ser	Pro					Asn	Trp	Ile	Lys	Ile	Glu	Val	Leu	Arg	Gly	Arg	Lys	Ala	Gly	Ser	Pro				
			210			215																																	
Leu	Glu	Ala	Gly	Pro	Leu	Gln	Asp	Phe	Trp	Gly	Asn	Pro	Thr	Ala	Ala					Leu	Glu	Ala	Gly	Pro	Leu	Gln	Asp	Phe	Trp	Gly	Asn	Pro	Thr	Ala	Ala				
					230					235																													
Ala	Leu	Met	Glu	Ala	Arg	Thr	Ala	Ala	Pro	Asp	Ala	Tyr	Ile	Ile	Ala					Ala	Leu	Met	Glu	Ala	Arg	Thr	Ala	Ala	Pro	Asp	Ala	Tyr	Ile	Ile	Ala				

<210> 66

&lt;211&gt; 379

&lt;212&gt; PRT

&lt;213&gt; Halobacterium sp. NRC-1

&lt;400&gt; 66

Met Gly Glu Ser Arg Tyr Asn Ser Ile Val Phe Pro Ser Leu Val Gln  
 1 5 10 15

Thr Arg Leu Met Thr Ala Gln Asp Ser Thr Gln Thr Glu Asp Arg Lys  
 20 25 30

Asp Asp His Leu Gln Ile Val Gln Glu Arg Asp Val Glu Thr Thr Gly  
 35 40 45

Thr Gly Phe Asp Asp Val His Leu Val His Asn Ala Leu Pro Glu Leu  
 50 55 60

Asp Tyr Asp Ala Ile Asp Pro Ser Ile Asp Phe Leu Gly His Asp Leu  
 65 70 75 80

Ser Ala Pro Ile Phe Ile Glu Ser Met Thr Gly Gly His His Asn Thr  
 85 90 95

Thr Glu Ile Asn Arg Ala Leu Ala Arg Ala Ala Ser Glu Thr Gly Ile  
 100 105 110

Ala Met Gly Leu Gly Ser Gln Arg Ala Gly Leu Glu Leu Asp Asp Glu  
 115 120 125

Arg Val Leu Glu Ser Tyr Thr Val Val Arg Asp Ala Ala Pro Asp Ala  
 130 135 140

Phe Ile Tyr Gly Asn Leu Gly Ala Ala Gln Leu Arg Glu Tyr Asp Ile  
 145 150 155 160

Glu Met Val Glu Gln Ala Val Glu Met Ile Asp Ala Asp Ala Leu Ala  
 165 170 175

Val His Leu Asn Phe Leu Gln Glu Ala Thr Gln Pro Glu Gly Asp Val  
 180 185 190

Asp Gly Arg Asn Cys Val Ala Ala Ile Glu Arg Val Ser Glu Ala Leu  
 195 200 205

Ser Val Pro Ile Ile Val Lys Glu Thr Gly Asn Gly Ile Ser Gly Glu  
 210 215 220

Thr Ala Arg Glu Leu Thr Ala Ala Gly Val Asp Ala Leu Asp Val Ala  
 225 230 235 240

Gly Lys Gly Gly Thr Thr Trp Ser Gly Ile Glu Ala Tyr Arg Ala Ala  
 245 250 255

Ala Ala Asn Ala Pro Arg Gln Lys Gln Ile Gly Thr Leu Phe Arg Glu

260                      265                      270  
 Trp Gly Ile Pro Thr Ala Ala Ser Thr Ile Glu Cys Val Ala Glu His  
       275                      280                      285  
 Asp Cys Val Ile Ala Ser Gly Gly Val Arg Thr Gly Leu Asp Val Ala  
       290                      295                      300  
 Lys Ala Ile Ala Leu Gly Ala Arg Ala Gly Gly Leu Ala Lys Pro Phe  
       305                      310                      315                      320  
 Leu Lys Pro Ala Thr Asp Gly Pro Asp Ala Val Ile Glu Arg Val Gly  
                              325                      330                      335  
 Asp Leu Ile Ala Glu Leu Arg Thr Ala Met Phe Val Thr Gly Ser Gly  
                              340                      345                      350  
 Ser Ile Asp Glu Leu Gln Gln Val Glu Tyr Val Leu His Gly Lys Thr  
                              355                      360                      365  
 Arg Glu Tyr Val Glu Gln Arg Thr Ser Ser Glu  
                              370                      375  
 <210> 67  
 <211> 317  
 <212> PRT  
 <213> Archaeoglobus fulgidus  
  
 <400> 67  
 Met Met Leu Ile His Lys Ala Leu Pro Glu Val Asp Tyr Trp Lys Ile  
       1                      5                      10                      15  
 Asp Thr Glu Ile Glu Phe Phe Gly Lys Lys Leu Ser Phe Pro Leu Leu  
                              20                      25                      30  
 Ile Ala Ser Met Thr Gly Gly His Pro Glu Thr Lys Glu Ile Asn Ala  
                              35                      40                      45  
 Arg Leu Gly Glu Ala Val Glu Glu Ala Gly Ile Gly Met Gly Val Gly  
                              50                      55                      60  
 Ser Gln Arg Ala Ala Ile Glu Asp Glu Ser Leu Ala Asp Ser Phe Thr  
       65                      70                      75                      80  
 Val Val Arg Glu Lys Ala Pro Asn Ala Phe Val Tyr Ala Asn Ile Gly  
                              85                      90                      95  
 Met Pro Gln Val Ile Glu Arg Gly Val Glu Ile Val Asp Arg Ala Val  
                              100                      105                      110  
 Glu Met Ile Asp Ala Asp Ala Val Ala Ile His Leu Asn Tyr Leu Gln

115                      120                      125  
 Glu Ala Ile Gln Pro Glu Gly Asp Leu Asn Ala Glu Lys Gly Leu Glu  
 130                      135                      140  
 Val Leu Glu Glu Val Cys Arg Ser Val Lys Val Pro Val Ile Ala Lys  
 145                      150                      155                      160  
 Glu Thr Gly Ala Gly Ile Ser Arg Glu Val Ala Val Met Leu Lys Arg  
 165                      170                      175  
 Ala Gly Val Ser Ala Ile Asp Val Gly Gly Lys Gly Gly Thr Thr Phe  
 180                      185                      190  
 Ser Gly Val Glu Val Tyr Arg Val Asn Asp Glu Val Ser Lys Ser Val  
 195                      200                      205  
 Gly Ile Asp Phe Trp Asp Trp Gly Leu Pro Thr Ala Phe Ser Ile Val  
 210                      215                      220  
 Asp Cys Arg Gly Ile Leu Pro Val Ile Ala Thr Gly Gly Leu Arg Ser  
 225                      230                      235                      240  
 Gly Leu Asp Val Ala Lys Ser Ile Ala Ile Gly Ala Glu Leu Gly Ser  
 245                      250                      255  
 Ala Ala Leu Pro Phe Leu Arg Ala Ala Val Glu Ser Ala Glu Lys Val  
 260                      265                      270  
 Arg Glu Glu Ile Glu Tyr Phe Arg Arg Gly Leu Lys Thr Ala Met Phe  
 275                      280                      285  
 Leu Thr Gly Cys Lys Asn Val Glu Glu Leu Lys Gly Leu Lys Val Phe  
 290                      295                      300  
 Val Ser Gly Arg Leu Lys Glu Trp Ile Asp Phe Arg Gly  
 305                      310                      315

&lt;210&gt; 68

&lt;211&gt; 370

&lt;212&gt; PRT

<213> *Pyrococcus abyssi*

&lt;400&gt; 68

Met Glu Glu Gln Thr Ile Leu Arg Lys Phe Glu His Ile Lys His Cys  
 1                      5                      10                      15  
 Leu Thr Lys Asn Val Glu Ala His Val Thr Asn Gly Phe Glu Asp Val  
 20                      25                      30  
 His Leu Ile His Lys Ser Leu Pro Glu Ile Asp Lys Asp Glu Ile Asp

35										40										45																											
Leu	Ser	Val	Lys	Phe	Leu	Gly	Arg	Lys	Phe	Asp	Tyr	Pro	Ile	Met	Ile	Leu	Gly	Arg	Lys	Phe	Asp	Tyr	Pro	Ile	Met	Ile	Leu	Gly	Arg	Lys	Phe	Asp	Tyr	Pro	Ile	Met	Ile										
50						55					60																																				
Thr	Gly	Met	Thr	Gly	Gly	Thr	Arg	Lys	Gly	Glu	Ile	Ala	Trp	Arg	Ile	Thr	Gly	Met	Thr	Gly	Gly	Thr	Arg	Lys	Gly	Glu	Ile	Ala	Trp	Arg	Ile	Thr	Gly	Met	Thr	Gly	Gly	Thr	Arg	Lys	Gly	Glu	Ile	Ala	Trp	Arg	Ile
65					70					75					80																																
Asn	Arg	Thr	Leu	Ala	Gln	Ala	Ala	Gln	Glu	Leu	Asn	Ile	Pro	Leu	Gly	Asn	Arg	Thr	Leu	Ala	Gln	Ala	Gln	Glu	Leu	Asn	Ile	Pro	Leu	Gly	Asn	Arg	Thr	Leu	Ala	Gln	Ala	Gln	Glu	Leu	Asn	Ile	Pro	Leu	Gly		
				85					90						95																																
Leu	Gly	Ser	Gln	Arg	Ala	Met	Ile	Glu	Lys	Pro	Glu	Thr	Trp	Glu	Ser	Leu	Gly	Ser	Gln	Arg	Ala	Met	Ile	Glu	Lys	Pro	Glu	Thr	Trp	Glu	Ser	Leu	Gly	Ser	Gln	Arg	Ala	Met	Ile	Glu	Lys	Pro	Glu	Thr	Trp	Glu	Ser
			100					105						110																																	
Tyr	Tyr	Val	Arg	Asp	Val	Ala	Pro	Asp	Val	Phe	Leu	Val	Gly	Asn	Leu	Tyr	Tyr	Val	Arg	Asp	Val	Ala	Pro	Asp	Val	Phe	Leu	Val	Gly	Asn	Leu	Tyr	Tyr	Val	Arg	Asp	Val	Ala	Pro	Asp	Val	Phe	Leu	Val	Gly	Asn	Leu
			115				120						125																																		
Gly	Ala	Pro	Gln	Phe	Gly	Arg	Asn	Ala	Lys	Lys	Arg	Tyr	Ser	Val	Asp	Gly	Ala	Pro	Gln	Phe	Gly	Arg	Asn	Ala	Lys	Lys	Arg	Tyr	Ser	Val	Asp	Gly	Ala	Pro	Gln	Phe	Gly	Arg	Asn	Ala	Lys	Lys	Arg	Tyr	Ser	Val	Asp
			130			135						140																																			
Glu	Val	Leu	Tyr	Ala	Ile	Glu	Lys	Ile	Glu	Ala	Asp	Ala	Ile	Ala	Ile	Glu	Val	Leu	Tyr	Ala	Ile	Glu	Lys	Ile	Glu	Ala	Asp	Ala	Ile	Ala	Ile	Glu	Val	Leu	Tyr	Ala	Ile	Glu	Lys	Ile	Glu	Ala	Asp	Ala	Ile	Ala	Ile
145				150					155					160																																	
His	Met	Asn	Pro	Leu	Gln	Glu	Ser	Ile	Gln	Pro	Glu	Gly	Asp	Thr	Thr	His	Met	Asn	Pro	Leu	Gln	Glu	Ser	Ile	Gln	Pro	Glu	Gly	Asp	Thr	Thr	His	Met	Asn	Pro	Leu	Gln	Glu	Ser	Ile	Gln	Pro	Glu	Gly	Asp	Thr	Thr
				165					170					175																																	
Phe	Ser	Gly	Val	Leu	Glu	Ala	Leu	Ala	Glu	Ile	Thr	Ser	Thr	Ile	Asp	Phe	Ser	Gly	Val	Leu	Glu	Ala	Leu	Ala	Glu	Ile	Thr	Ser	Thr	Ile	Asp	Phe	Ser	Gly	Val	Leu	Glu	Ala	Leu	Ala	Glu	Ile	Thr				

340 345 350

Trp Leu Leu Gln Arg Ile Asp Leu Asn Ser Tyr Leu Arg Ala Arg Phe  
355 360 365

Lys Met  
370

<210> 69

<211> 371

<212> PRT

<213> *Pyrococcus horikoshii*

<400> 69

Met Lys Glu Glu Leu Thr Ile Leu Arg Lys Phe Glu His Ile Glu His  
1 5 10 15

Cys Leu Lys Arg Asn Val Glu Ala His Val Ser Asn Gly Phe Glu Asp  
20 25 30

Val Tyr Phe Val His Lys Ser Leu Pro Glu Ile Asp Lys Asp Glu Ile  
35 40 45

Asp Leu Thr Val Thr Glu Phe Leu Gly Arg Lys Phe Asp Tyr Pro Ile Met  
50 55 60

Ile Thr Gly Met Thr Gly Gly Thr Arg Arg Glu Glu Ile Ala Gly Lys  
65 70 75 80

Ile Asn Arg Thr Leu Ala Met Ala Ala Glu Glu Leu Asn Ile Pro Phe  
85 90 95

Gly Val Gly Ser Gln Arg Ala Met Ile Glu Lys Pro Glu Thr Trp Glu  
100 105 110

Ser Tyr Tyr Val Arg Asp Val Ala Pro Asp Ile Phe Leu Ile Gly Asn  
115 120 125

Leu Gly Ala Pro Gln Phe Gly Lys Asn Ala Lys Lys Arg Tyr Ser Val  
130 135 140

Lys Glu Val Leu Tyr Ala Ile Glu Lys Ile Glu Ala Asp Ala Ile Ala  
145 150 155 160

Ile His Met Asn Pro Leu Gln Glu Ser Val Gln Pro Glu Gly Asp Thr  
165 170 175

Thr Tyr Ala Gly Val Leu Glu Ala Leu Ala Glu Ile Lys Ser Ser Ile  
180 185 190

Asn Tyr Pro Val Ile Ala Lys Glu Thr Gly Ala Gly Val Ser Lys Glu

195                      200                      205  
 Val Ala Ile Glu Leu Glu Ser Val Gly Ile Asp Ala Ile Asp Ile Ser  
 210                      215                      220  
 Gly Leu Gly Gly Thr Ser Trp Ser Ala Val Glu Tyr Tyr Arg Ala Lys  
 225                      230                      235                      240  
 Asp Ser Glu Lys Arg Lys Ile Ala Leu Lys Phe Trp Asp Trp Gly Ile  
 245                      250                      255  
 Lys Thr Ala Ile Ser Leu Ala Glu Val Arg Trp Ala Thr Asn Leu Pro  
 260                      265                      270  
 Ile Ile Ala Ser Gly Gly Met Arg Asp Gly Val Met Met Ala Lys Ala  
 275                      280                      285  
 Leu Ala Met Gly Ala Ser Leu Val Gly Ile Ala Leu Pro Val Leu Arg  
 290                      295                      300  
 Pro Ala Ala Arg Gly Asp Val Glu Gly Val Val Arg Ile Ile Arg Gly  
 305                      310                      315                      320  
 Tyr Ala Glu Glu Ile Lys Asn Val Met Phe Leu Val Gly Ala Arg Asn  
 325                      330                      335  
 Ile Arg Glu Leu Arg Arg Val Pro Leu Val Ile Thr Gly Phe Val Arg  
 340                      345                      350  
 Glu Trp Leu Leu Gln Arg Ile Asp Leu Asn Ser Tyr Leu Arg Ser Arg  
 355                      360                      365  
 Phe Lys His  
 370  
 <210> 70  
 <211> 349  
 <212> PRT  
 <213> Methanobacterium thermoautotrophicum  
  
 <400> 70  
 Met Ile Ser Asp Arg Lys Leu Glu His Leu Ile Leu Cys Ala Ser Cys  
 1                      5                      10                      15  
 Asp Val Glu Tyr Arg Lys Lys Thr Gly Phe Glu Asp Ile Glu Ile Val  
 20                      25                      30  
 His Arg Ala Ile Pro Glu Ile Asn Lys Glu Lys Ile Asp Ile Ser Leu  
 35                      40                      45  
 Asp Phe Leu Gly Arg Glu Leu Ser Ser Pro Val Met Ile Ser Ala Ile



50                                      55                                      60  
 Thr Gly Gly His Pro Ala Ser Met Lys Ile Asn Arg Glu Leu Ala Arg  
 65                                      70                                      75                                      80  
 Ala Ala Glu Lys Leu Gly Ile Ala Leu Gly Leu Gly Ser Gln Arg Ala  
                                     85                                      90                                      95  
 Gly Val Glu His Pro Glu Leu Glu Gly Thr Tyr Thr Ile Ala Arg Glu  
                                     100                                      105                                      110  
 Glu Ala Pro Ser Ala Met Leu Ile Gly Asn Ile Gly Ser Ser His Ile  
                                     115                                      120                                      125  
 Glu Tyr Ala Glu Arg Ala Val Glu Met Ile Asp Ala Asp Ala Leu Ala  
 130                                      135                                      140  
 Val His Leu Asn Pro Leu Gln Glu Ser Ile Gln Pro Gly Gly Asp Val  
 145                                      150                                      155                                      160  
 Asp Ser Ser Gly Ala Leu Glu Ser Ile Ser Ala Ile Val Glu Ser Val  
                                     165                                      170                                      175  
 Asp Val Pro Val Met Val Lys Glu Thr Gly Ala Gly Ile Cys Ser Glu  
                                     180                                      185                                      190  
 Asp Ala Ile Glu Leu Glu Ser Cys Gly Val Ser Ala Ile Asp Val Ala  
                                     195                                      200                                      205  
 Gly Ala Gly Gly Thr Ser Trp Ala Ala Val Glu Thr Tyr Arg Ala Asp  
 210                                      215                                      220  
 Asp Arg Tyr Leu Gly Glu Leu Phe Trp Asp Trp Gly Ile Pro Thr Ala  
 225                                      230                                      235                                      240  
 Ala Ser Thr Val Glu Val Val Glu Ser Val Ser Ile Pro Val Ile Ala  
                                     245                                      250                                      255  
 Ser Gly Gly Ile Arg Ser Gly Ile Asp Ala Ala Lys Ala Ile Ser Leu  
                                     260                                      265                                      270  
 Gly Ala Glu Met Val Gly Ile Ala Leu Pro Val Leu Glu Ala Ala Gly  
                                     275                                      280                                      285  
 His Gly Tyr Arg Glu Val Ile Lys Val Ile Glu Gly Phe Asn Glu Ala  
 290                                      295                                      300  
 Leu Arg Thr Ala Met Tyr Leu Ala Gly Ala Glu Thr Leu Asp Asp Leu  
 305                                      310                                      315                                      320  
 Lys Lys Ser Pro Val Ile Ile Thr Gly His Thr Gly Glu Trp Leu Asn  
                                     325                                      330                                      335  
 Gln Arg Gly Phe Glu Thr Lys Lys Tyr Ala Arg Arg Ser  
                                     340                                      345

&lt;210&gt; 71

&lt;211&gt; 359

&lt;212&gt; PRT

&lt;213&gt; Methanococcus jannaschii

&lt;400&gt; 71

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Met Val Asn Asn Arg Asn Glu Ile Glu Val Arg Lys Leu Glu His Ile
 1           5           10           15

Phe Leu Cys Ser Tyr Cys Asn Val Glu Tyr Glu Lys Thr Thr Leu Leu
 20           25           30

Glu Asp Ile Glu Leu Ile His Lys Gly Thr Cys Gly Ile Asn Phe Asn
 35           40           45

Asp Ile Glu Thr Glu Ile Glu Leu Phe Gly Lys Lys Leu Ser Ala Pro
 50           55           60

Ile Ile Val Ser Gly Met Thr Gly Gly His Ser Lys Ala Lys Glu Ile
 65           70           75           80

Asn Lys Asn Ile Ala Lys Ala Val Glu Glu Leu Gly Leu Gly Met Gly
 85           90           95

Val Gly Ser Gln Arg Ala Ala Ile Val Asn Asp Glu Leu Ile Asp Thr
100           105           110

Tyr Ser Ile Val Arg Asp Tyr Thr Asn Asn Leu Val Ile Gly Asn Leu
115           120           125

Gly Ala Val Asn Phe Ile Val Asp Asp Trp Asp Glu Glu Ile Ile Asp
130           135           140

Lys Ala Ile Glu Met Ile Asp Ala Asp Ala Ile Ala Ile His Phe Asn
145           150           155           160

Pro Leu Gln Glu Ile Ile Gln Pro Glu Gly Asp Leu Asn Phe Lys Asn
165           170           175

Leu Tyr Lys Leu Lys Glu Ile Ile Ser Asn Tyr Lys Lys Ser Tyr Lys
180           185           190

Asn Ile Pro Phe Ile Ala Lys Gln Val Gly Glu Gly Phe Ser Lys Glu
195           200           205

Asp Ala Leu Ile Leu Lys Asp Ile Gly Phe Asp Ala Ile Asp Val Gln
210           215           220

Gly Ser Gly Gly Thr Ser Trp Ala Lys Val Glu Ile Tyr Arg Val Lys
225           230           235           240

Glu Glu Glu Ile Lys Arg Leu Ala Glu Lys Phe Ala Asn Trp Gly Ile

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[illegible]

115                      120                      125  
 Gln Asp Lys Asp Ala Val Ser Asn Arg Asp Ile Ala Tyr Ile Tyr Asp  
 130                      135                      140  
 Leu Ile Lys Ala Asp Phe Leu Ala Val His Phe Asn Phe Leu Gln Glu  
 145                      150                      155                      160  
 Met Val Gln Pro Glu Gly Asp Arg Asn Ser Lys Gly Val Ile Asp Arg  
 165                      170                      175  
 Ile Lys Asp Leu Ser Gly Ser Phe Asn Ile Ile Ala Lys Glu Thr Gly  
 180                      185                      190  
 Ser Gly Phe Ser Arg Arg Thr Ala Glu Arg Leu Ile Asp Ala Gly Val  
 195                      200                      205  
 Lys Ala Ile Glu Val Ser Gly Val Ser Gly Thr Thr Phe Ala Ala Val  
 210                      215                      220  
 Glu Tyr Tyr Arg Ala Arg Lys Glu Asn Asn Leu Glu Lys Met Arg Ile  
 225                      230                      235                      240  
 Gly Glu Thr Phe Trp Asn Trp Gly Ile Pro Ser Pro Ala Ser Val Tyr  
 245                      250                      255  
 Tyr Cys Ser Asp Leu Ala Pro Val Ile Gly Ser Gly Gly Leu Arg Asn  
 260                      265                      270  
 Gly Leu Asp Leu Ala Lys Ala Ile Ala Met Gly Ala Thr Ala Gly Gly  
 275                      280                      285  
 Phe Ala Arg Ser Leu Leu Lys Asp Ala Asp Thr Asp Pro Glu Met Leu  
 290                      295                      300  
 Met Lys Asn Ile Glu Leu Ile Gln Arg Glu Phe Arg Val Ala Leu Phe  
 305                      310                      315                      320  
 Leu Thr Gly Asn Lys Asn Val Tyr Glu Leu Lys Phe Thr Lys Lys Val  
 325                      330                      335  
 Ile Val Asp Pro Leu Arg Ser Trp Leu Glu Ala Lys  
 340                      345  
 <210> 73  
 <211> 357  
 <212> PRT  
 <213> Leishmania major  
 <400> 73  
 Met Ser Ser Arg Asp Cys Thr Val Asp Arg Glu Ala Ala Val Gln Lys

1	5							10					15					
Arg	Lys	Lys	Asp	His	Ile	Asp	Ile	Cys	Leu	His	Gln	Asp	Val	Glu	Pro			
20							25							30				
His	Lys	Arg	Arg	Thr	Ser	Ile	Trp	Asn	Lys	Tyr	Thr	Leu	Pro	Tyr	Lys			
35							40							45				
Ala	Leu	Pro	Glu	Val	Asp	Leu	Gln	Lys	Ile	Asp	Thr	Ser	Cys	Glu	Phe			
50							55							60				
Met	Gly	Lys	Arg	Ile	Ser	Phe	Pro	Phe	Phe	Ile	Ser	Ser	Met	Thr	Gly			
65							70							75				
Gly	Glu	Ala	His	Gly	Arg	Val	Ile	Asn	Glu	Asn	Leu	Ala	Lys	Ala	Cys			
85							90							95				
Glu	Ala	Glu	Lys	Ile	Pro	Phe	Gly	Leu	Gly	Ser	Met	Arg	Ile	Ile	Asn			
100							105							110				
Arg	Tyr	Ala	Ser	Ala	Val	His	Thr	Phe	Asn	Val	Lys	Glu	Phe	Cys	Pro			
115							120							125				
Ser	Val	Pro	Met	Leu	Ala	Asn	Ile	Gly	Leu	Val	Gln	Leu	Asn	Tyr	Gly			
130							135							140				
Phe	Gly	Pro	Lys	Glu	Val	Asn	Asn	Leu	Val	Asn	Ser	Val	Arg	Ala	Asp			
145							150							155				
Gly	Leu	Cys	Ile	His	Leu	Asn	His	Thr	Gln	Glu	Val	Cys	Gln	Pro	Glu			
165							170							175				
Gly	Asp	Thr	Asn	Phe	Glu	Gly	Leu	Ile	Glu	Lys	Leu	Arg	Gln	Leu	Leu			
180							185							190				
Pro	His	Ile	Lys	Val	Pro	Val	Leu	Val	Lys	Gly	Val	Gly	His	Gly	Ile			
195							200							205				
Asp	Tyr	Glu	Ser	Met	Val	Ala	Ile	Lys	Ala	Ser	Gly	Val	Lys	Tyr	Val			
210							215							220				
Asp	Val	Ser	Gly	Cys	Gly	Gly	Thr	Ser	Trp	Ala	Trp	Ile	Glu	Gly	Arg			
225							230							235				
Arg	Gln	Pro	Tyr	Lys	Ala	Glu	Glu	Glu	Asn	Ile	Gly	Tyr	Leu	Leu	Arg			
245							250							255				
Asp	Ile	Gly	Val	Pro	Thr	Asp	Val	Cys	Leu	Arg	Glu	Ser	Ala	Pro	Leu			
260							265							270				
Thr	Val	Asn	Gly	Asp	Leu	His	Leu	Ile	Ala	Gly	Gly	Gly	Ile	Arg	Asn			
275							280							285				
Gly	Met	Asp	Val	Ala	Lys	Ala	Leu	Met	Met	Gly	Ala	Glu	Tyr	Ala	Thr			
290							295							300				
Ala	Ala	Met	Pro	Phe	Leu	Ala	Ala	Ala	Leu	Glu	Ser	Ser	Glu	Ala	Val			





[illegible]



325										330										335																											
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340										345										350																											
Glu	Gln	Val	Gln	Leu	Asp	Asp	Asn	Gly	Asn	Ala	Asn	Phe	Asp	Ile	Tyr	Glu	Gln	Val	Gln	Leu	Asp	Asp	Asn	Gly	Asn	Ala	Asn	Phe	Asp	Ile	Tyr	Glu	Gln	Val	Gln	Leu	Asp	Asp	Asn	Gly	Asn	Ala	Asn	Phe	Asp	Ile	Tyr
355										360										365																											
Leu	Thr	Gly	Lys	Phe	Ala	Leu	Thr	Ala	Ile	Lys	Glu	His	Gln	Arg	Ile	Leu	Thr	Gly	Lys	Phe	Ala	Leu	Thr	Ala	Ile	Lys	Glu	His	Gln	Arg	Ile	Leu	Thr	Gly	Lys	Phe	Ala	Leu	Thr	Ala	Ile	Lys	Glu	His	Gln	Arg	Ile
370										375										380																											
Tyr	His	Thr	Asn	Asp	Lys	Asn										Tyr	His	Thr	Asn	Asp	Lys	Asn										Tyr	His	Thr	Asn	Asp	Lys	Asn									
385										390																																					
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<211> 383																																															
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1										5										10																											
Ile	Asp	Met	Thr	Ala	Leu	Ala	Glu	Ala	Arg	Asn	Val	Asp	Pro	Gly	Lys	Ile	Asp	Met	Thr	Ala	Leu	Ala	Glu	Ala	Arg	Asn	Val	Asp	Pro	Gly	Lys	Ile	Asp	Met	Thr	Ala	Leu	Ala	Glu	Ala	Arg	Asn	Val	Asp	Pro	Gly	Lys
20										25										30																											
Phe	His	Ile	Gly	Ile	Gly	Gln	Asp	Gln	Met	Ala	Val	Asn	Pro	Ile	Ser	Phe	His	Ile	Gly	Ile	Gly	Gln	Asp	Gln	Met	Ala	Val	Asn	Pro	Ile	Ser	Phe	His	Ile	Gly	Ile	Gly	Gln	Asp	Gln	Met	Ala	Val	Asn	Pro	Ile	Ser
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50										55										60																											
Lys	Glu	Asp	Lys	Glu	Ala	Ile	Asp	Met	Val	Ile	Val	Gly	Thr	Glu	Ser	Lys	Glu	Asp	Lys	Glu	Ala	Ile	Asp	Met	Val	Ile	Val	Gly	Thr	Glu	Ser	Lys	Glu	Asp	Lys	Glu	Ala	Ile	Asp	Met	Val	Ile	Val	Gly	Thr	Glu	Ser
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85										90										95																											
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100										105										110																											
Gly	Ala	Thr	Ala	Gly	Leu	Gln	Leu	Ala	Lys	Asn	His	Val	Ala	Leu	His	Gly	Ala	Thr	Ala	Gly	Le																										

165										170					175					
Met	Leu	Thr	Gln	Asp	Ile	Tyr	Asp	Phe	Trp	Arg	Pro	Thr	Gly	His	Pro					
180										185					190					
Tyr	Pro	Met	Val	Asp	Gly	Pro	Leu	Ser	Asn	Glu	Thr	Tyr	Ile	Gln	Ser					
195										200					205					
Phe	Ala	Gln	Val	Trp	Asp	Glu	His	Lys	Lys	Arg	Thr	Gly	Leu	Asp	Phe					
210										215					220					
Ala	Asp	Tyr	Asp	Ala	Leu	Ala	Phe	His	Ile	Pro	Tyr	Thr	Lys	Met	Gly					
225										230					235					
Lys	Lys	Ala	Leu	Leu	Ala	Lys	Ile	Ser	Asp	Gln	Thr	Glu	Ala	Glu	Gln					
245										250					255					
Glu	Arg	Ile	Leu	Ala	Arg	Tyr	Glu	Glu	Ser	Ile	Ile	Tyr	Ser	Arg	Arg					
260										265					270					
Val	Gly	Asn	Leu	Tyr	Thr	Gly	Ser	Leu	Tyr	Leu	Gly	Leu	Ile	Ser	Leu					
275										280					285					
Leu	Glu	Asn	Ala	Thr	Thr	Leu	Thr	Ala	Gly	Asn	Gln	Ile	Gly	Leu	Phe					
290										295					300					
Ser	Tyr	Gly	Ser	Gly	Ala	Val	Ala	Glu	Phe	Phe	Thr	Gly	Glu	Leu	Val					
305										310					315					
Ala	Gly	Tyr	Gln	Asn	His	Leu	Gln	Lys	Glu	Thr	His	Leu	Ala	Leu	Leu					
325										330					335					
Asp	Asn	Arg	Thr	Glu	Leu	Ser	Ile	Ala	Glu	Tyr	Glu	Ala	Met	Phe	Ala					
340										345					350					
Glu	Thr	Leu	Asp	Thr	Asp	Ile	Asp	Gln	Thr	Leu	Glu	Asp	Glu	Leu	Lys					
355										360					365					
Tyr	Ser	Ile	Ser	Ala	Ile	Asn	Asn	Thr	Val	Arg	Ser	Tyr	Arg	Asn						
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<210> 77

&lt;211&gt; 384

&lt;212&gt; PRT

<213> Enterococcus faecium

<400> 77

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Leu Asp Met Thr Glu Leu Ala Glu Ser Arg Gly Asp Asp Pro Ala Lys

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Tyr	His	Ile	Gly	Ile	Gly	Gln
	35					40
				Asp	Gln	Met
					Ala	Val
					Asn	Arg
					Ala	Asn
Glu	Asp	Ile	Ile	Thr	Leu	Gly
	50					55
					Ala	Asn
					Ala	Ala
					Ser	Lys
					Ile	Val
					Thr	
Glu	Lys	Asp	Arg	Glu	Leu	Ile
	65				70	
						75
					Met	Val
					Ile	Val
					Gly	Thr
					Glu	Ser
						80
Gly	Ile	Asp	His	Ser	Lys	Ala
						85
					Ser	Ala
					Val	Ile
					Ile	His
					His	Leu
						95
Lys	Ile	Gln	Ser	Phe	Ala	Arg
						100
					Ser	Phe
					Glu	Val
					Lys	Glu
					Ala	Cys
					Tyr	
						110
Gly	Gly	Thr	Ala	Ala	Leu	His
						115
					Met	Ala
					Lys	Glu
					Tyr	Val
					Lys	Asn
					His	
						125
Pro	Glu	Arg	Lys	Val	Leu	Val
						130
					Ile	Ala
					Ser	Asp
					Ile	Ala
					Arg	Tyr
					Gly	
						140
Leu	Ala	Ser	Gly	Gly	Glu	Val
						145
					Thr	Gln
					Gly	Val
					Gly	Ala
					Val	Ala
					Met	
						160
Met	Ile	Thr	Gln	Asn	Pro	Arg
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					Ile	Leu
					Ser	Ile
					Glu	Asp
					Asp	Ser
					Val	
						175
Phe	Leu	Thr	Glu	Asp	Ile	Tyr
						180
					Asp	Phe
					Trp	Arg
					Pro	Asp
					Tyr	Ser
					Glu	
						190
Phe	Pro	Val	Val	Asp	Gly	Pro
						195
					Leu	Ser
					Asn	Ser
					Thr	Tyr
					Ile	Glu
					Ser	
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Phe	Gln	Lys	Val	Trp	Asn	Arg
						210
					His	Lys
					Glu	Leu
					Ser	Gly
					Arg	Gly
					Leu	
						220
Glu	Asp	Tyr	Gln	Ala	Ile	Ala
						225
					Phe	His
					Ile	Pro
					Tyr	Thr
					Lys	Met
					Gly	
						240
Lys	Lys	Ala	Leu	Gln	Ser	Val
						245
					Leu	Asp
					Gln	Thr
					Asp	Glu
					Asn	Gln
						255
Glu	Arg	Leu	Met	Ala	Arg	Tyr
						260
					Glu	Ser
					Ile	Arg
					Tyr	Ser
					Arg	Arg
						270
Ile	Gly	Asn	Leu	Tyr	Thr	Gly
						275
					Ser	Leu
					Tyr	Leu
					Gly	Leu
					Thr	Ser
					Leu	
						285
Leu	Glu	Asn	Ser	Lys	Ser	Leu
						290
					Gln	Pro
					Gly	Asp
					Arg	Ile
					Gly	Leu
					Phe	
						300
Ser	Tyr	Gly	Ser	Gly	Ala	Val
						310
					Ser	Glu
					Phe	Phe
					Thr	Gly
					Tyr	Leu
					Glu	
						320
Glu	Asn	Tyr	Gln	Glu	Tyr	Leu
					Phe	Ala
					Gln	Ser
					His	Gln
					Glu	Met
					Leu	

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      325              330              335
Asp Ser Arg Thr Arg Ile Thr Val Asp Glu Tyr Glu Thr Ile Phe Ser
      340              345              350
Glu Thr Leu Pro Glu His Gly Glu Cys Ala Glu Tyr Thr Ser Asp Val
      355              360              365
Pro Phe Ser Ile Thr Lys Ile Glu Asn Asp Ile Arg Tyr Tyr Lys Ile
      370              375              380

<210>   78
<211>   388
<212>    PRT
<213>   Staphylococcus haemolyticus

<400>   78
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          20                25                30
Phe Leu Ile Gly Ile Gly Gln Thr Gln Met Ala Val Ser Pro Val Ser
        35                40                45
Gln Asp Ile Val Ser Met Gly Ala Asn Ala Ala Lys Asp Ile Ile Thr
 50                55                60
Asp Asp Asp Lys Lys His Ile Gly Met Val Ile Val Ala Thr Glu Ser
65                70                75
Ala Ile Asp Asn Ala Lys Ala Ala Val Gln Ile His Asn Leu Leu
          85                90                95
Gly Val Gln Pro Phe Ala Arg Cys Phe Glu Met Lys Glu Ala Cys Tyr
          100               105               110
Ala Ala Thr Pro Ala Ile Gln Leu Ala Lys Asp Tyr Ile Glu Lys Arg
       115               120               125
Pro Asn Glu Lys Val Leu Val Ile Ala Ser Asp Thr Ala Arg Tyr Gly
       130               135               140
Ile Gln Ser Gly Gly Glu Pro Thr Gln Gly Ala Gly Ala Val Ala Met
145               150               155               160
Leu Ile Ser Ser Asn Asn Pro Ser Ile Leu Glu Leu Asn Asp Asp Ala Val
          165               170               175
Ala Tyr Thr Glu Asp Val Tyr Asp Phe Trp Arg Pro Thr Gly His Lys

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180										185					190				
Tyr	Pro	Leu	Val	Ala	Gly	Ala	Leu	Ser	Lys	Asp	Ala	Tyr	Ile	Lys	Ser				
195						200					205								
Phe	Gln	Glu	Ser	Trp	Asn	Glu	Tyr	Ala	Arg	Arg	Glu	Asp	Lys	Thr	Leu				
210						215					220								
Ser	Asp	Phe	Glu	Ser	Leu	Cys	Phe	His	Val	Pro	Phe	Thr	Lys	Met	Gly				
225						230					235								
Lys	Lys	Ala	Leu	Asp	Ser	Ile	Ile	Asn	Asp	Ala	Asp	Glu	Thr	Thr	Gln				
245						250					255								
Glu	Arg	Leu	Thr	Ser	Gly	Tyr	Glu	Asp	Ala	Val	Tyr	Tyr	Asn	Arg	Tyr				
260						265					270								
Val	Gly	Asn	Ile	Tyr	Thr	Gly	Ser	Leu	Tyr	Leu	Ser	Leu	Ile	Ser	Leu				
275						280					285								
Leu	Glu	Asn	Arg	Ser	Leu	Lys	Gly	Gly	Gln	Thr	Ile	Gly	Leu	Phe	Ser				
290						295					300								
Tyr	Gly	Ser	Gly	Ser	Val	Gly	Glu	Phe	Phe	Ser	Ala	Thr	Leu	Val	Glu				
305						310					315								
Gly	Tyr	Glu	Lys	Gln	Leu	Asp	Ile	Glu	Gly	His	Lys	Ala	Leu	Leu	Asn				
325						330					335								
Glu	Arg	Gln	Glu	Val	Ser	Val	Glu	Asp	Tyr	Glu	Ser	Phe	Phe	Lys	Arg				
340						345					350								
Phe	Asp	Asp	Leu	Glu	Phe	Asp	His	Ala	Thr	Glu	Gln	Thr	Asp	Asp	Asp				
355						360					365								
Lys	Ser	Ile	Tyr	Tyr	Leu	Glu	Asn	Ile	Gln	Asp	Asp	Ile	Arg	Gln	Tyr				
370						375					380								
His Ile Pro Lys																			
385																			

<210> 79

<211> 388

&lt;212&gt; PRT

<213> Staphylococcus epidermis

&lt;400&gt; 79

Met Asn Ile Gly Ile Asp Lys Ile Ser Phe Tyr Val Pro Lys Tyr Tyr  
1 5 10 15

Val Asp Met Ala Lys Leu Ala Glu Ala Arg Gln Val Asp Pro Asn Lys

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Phe	Leu	Ile	Gly	Ile	Gly	Gln	Thr	Glu	Met	Thr	Val	Ser	Pro	Val	Asn														
35										40										45									
Gln	Asp	Ile	Val	Ser	Met	Gly	Ala	Asn	Ala	Ala	Lys	Asp	Ile	Ile	Thr														
50										55										60									
Glu	Glu	Asp	Lys	Lys	Asn	Ile	Gly	Met	Val	Ile	Val	Ala	Thr	Glu	Ser														
65										70										75									
Ala	Ile	Asp	Asn	Ala	Lys	Ala	Ala	Ala	Val	Gln	Ile	His	His	Leu	Leu														
										85										90									
Gly	Ile	Gln	Pro	Phe	Ala	Arg	Cys	Phe	Glu	Met	Lys	Glu	Ala	Cys	Tyr														
										100										105									
Ala	Ala	Thr	Pro	Ala	Ile	Gln	Leu	Ala	Lys	Asp	Tyr	Leu	Ala	Gln	Arg														
										115										120									
Pro	Asn	Glu	Lys	Val	Leu	Val	Ile	Ala	Ser	Asp	Thr	Ala	Arg	Tyr	Gly														
										130										135									
Ile	His	Ser	Gly	Gly	Glu	Pro	Thr	Gln	Gly	Ala	Gly	Ala	Val	Ala	Met														
										145										150									
Met	Ile	Ser	His	Asp	Pro	Ser	Ile	Leu	Lys	Leu	Asn	Asp	Asp	Ala	Val														
										165										170									
Ala	Tyr	Thr	Glu	Asp	Val	Tyr	Asp	Phe	Trp	Arg	Pro	Thr	Gly	His	Gln														
										180										185									
Tyr	Pro	Leu	Val	Ala	Gly	Ala	Leu	Ser	Lys	Asp	Ala	Tyr	Ile	Lys	Ser														
										195										200									
Phe	Gln	Glu	Ser	Trp	Asn	Glu	Tyr	Ala	Arg	Arg	His	Asn	Lys	Thr	Leu														
										210										215									
Ala	Asp	Phe	Ala	Ser	Leu	Cys	Phe	His	Val	Pro	Phe	Thr	Lys	Met	Gly														
										225										230									
Gln	Lys	Ala	Leu	Asp	Ser	Ile	Ile	Asn	His	Ala	Asp	Glu	Thr	Thr	Gln														
										245										250									
Asp	Arg	Leu	Asn	Ser	Ser	Tyr	Gln	Asp	Ala	Val	Asp	Tyr	Asn	Arg	Tyr														
										260										265									
Val	Gly	Asn	Ile	Tyr	Thr	Gly	Ser	Leu	Tyr	Leu	Ser	Leu	Ile	Ser	Leu														
										275										280									
Leu	Glu	Thr	Arg	Asp	Leu	Lys	Gly	Gly	Gln	Thr	Ile	Gly	Leu	Phe	Ser														
										290										295									
Tyr	Gly	Ser	Gly	Ser	Val	Gly	Glu	Phe	Phe	Ser	Gly	Thr	Leu	Val	Asp														
										305										310									
Gly	Phe	Lys	Glu	Gln	Leu	Asp	Val	Glu	Arg	His	Lys	Ser	Leu	Leu	Asn														

[illegible]

[illegible]



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Phe	Leu	Ile	Gly	Ile	Gly	Gln	Ser	Gln	Met	Ala	Val	Ser	Pro	Val	Ser				
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Gln	Asp	Ile	Val	Ser	Met	Gly	Ala	Asn	Ala	Ala	Gln	Pro	Ile	Leu	Ser				
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Glu	Gln	Asp	Lys	Lys	Asp	Ile	Thr	Met	Val	Ile	Val	Ala	Thr	Glu	Ser				
65					70					75									
Ala	Ile	Asp	Ser	Ala	Lys	Ala	Ser	Ala	Val	Gln	Ile	His	His	Leu	Leu				
85					90					95									
Gly	Ile	Gln	Pro	Phe	Ala	Arg	Cys	Phe	Glu	Met	Lys	Glu	Ala	Cys	Tyr				
100					105					110									
Ala	Ala	Thr	Pro	Ala	Ile	Gln	Leu	Ala	Lys	Asp	Tyr	Leu	Val	Pro	Arg				
115					120					125									
Pro	Lys	Glu	Lys	Val	Leu	Val	Ile	Ala	Ser	Asp	Thr	Ala	Arg	Tyr	Gly				
130					135					140									
Leu	Asn	Ser	Gly	Gly	Glu	Pro	Thr	Gln	Gly	Ala	Gly	Ala	Val	Ala	Met				
145					150					155									
Val	Ile	Ser	His	Asn	Pro	Ser	Ile	Leu	Glu	Leu	His	Asp	Asp	Ser	Val				
165					170					175									
Ala	Tyr	Thr	Glu	Asp	Val	Tyr	Asp	Phe	Trp	Arg	Pro	Ser	Gly	Glu	Ile				
180					185					190									
Tyr	Pro	Leu	Val	Ala	Gly	Lys	Leu	Ser	Lys	Asp	Ala	Tyr	Ile	Lys	Ser				
195					200					205									
Phe	Gln	Glu	Ser	Trp	Asn	Glu	Tyr	Ala	Lys	Arg	His	His	Lys	Ser	Leu				
210					215					220									
Ser	Asp	Phe	Ala	Ala	Leu	Cys	Phe	His	Val	Pro	Phe	Thr	Lys	Met	Gly				
225					230					235									
Gln	Lys	Ala	Leu	Asp	Ser	Ile	Leu	Thr	Asp	Ser	Ala	Ser	Glu	Asp	Thr				
245					250					255									
Gln	Ala	Arg	Leu	Asn	Glu	Gly	Tyr	Lys	Ser	Ala	Thr	Asp	Tyr	Asn	Arg				
260					265					270									
Tyr	Val	Gly	Asn	Val	Tyr	Thr	Gly	Ser	Leu	Tyr	Leu	Ser	Leu	Ile	Ser				
275					280					285									
Leu	Leu	Glu	Asn	His	Lys	Leu	Asn	Gly	Gly	Asp	Asn	Ile	Gly	Leu	Phe				
290					295					300									
Ser	Tyr	Gly	Ser	Gly	Ser	Val	Gly	Glu	Phe	Phe	Ser	Ala	Thr	Leu	Val				

305                    310                    315                    320  
 Asp Asn Tyr Gln Asp His Leu Asp Val Lys Ala His Lys Ala Met Leu  
                          325                    330                    335  
 Asp Asn Arg Lys Ala Leu Ser Val Glu Glu Tyr Glu Lys Phe Phe Asn  
                          340                    345                    350  
 Arg Phe Asp Asn Leu Glu Phe Asp Thr Glu Thr Glu Leu Glu Val Glu  
                          355                    360                    365  
 Pro Lys Gly Asn Phe Tyr Leu Lys Glu Ile Ser Asp Asn Ile Arg Tyr  
                          370                    375                    380  
 Tyr Asp Thr Val Lys  
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 <212> PRT  
 <213> Streptomyces sp. CL190  
  
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                          20                    25                    30  
 Gly Lys Tyr His Val Gly Ile Gly Gln Gln Ser Met Ser Val Pro Ala  
                          35                    40                    45  
 Ala Asp Glu Asp Ile Val Thr Met Ala Ala Thr Ala Ala Arg Pro Ile  
                          50                    55                    60  
 Ile Glu Arg Asn Gly Lys Ser Arg Ile Arg Thr Val Val Phe Ala Thr  
 65                    70                    75                    80  
 Glu Ser Ser Ile Asp Gln Ala Lys Ala Gly Gly Val Tyr Val His Ser  
                          85                    90                    95  
 Leu Leu Gly Leu Glu Ser Ala Cys Arg Val Val Glu Leu Lys Gln Ala  
                          100                    105                    110  
 Cys Tyr Gly Ala Thr Ala Ala Leu Gln Phe Ala Ile Gly Leu Val Arg  
                          115                    120                    125  
 Arg Asp Pro Ala Gln Gln Val Leu Val Ile Ala Ser Asp Val Ser Lys  
                          130                    135                    140  
 Tyr Glu Leu Asp Ser Pro Gly Glu Ala Thr Gln Gly Ala Ala Ala Val

145                      150                      155                      160  
 Ala Met Leu Val Gly Ala Asp Pro Ala Leu Leu Arg Ile Glu Glu Pro  
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 Ser Gly Leu Phe Thr Ala Asp Val Met Asp Phe Trp Arg Pro Asn Tyr  
                                  180                                   185                                   190  
 Leu Thr Thr Ala Leu Val Asp Gly Gln Glu Ser Ile Asn Ala Tyr Leu  
                                  195                                   200                                   205  
 Gln Ala Val Glu Gly Ala Trp Lys Asp Tyr Ala Glu Gln Asp Gly Arg  
                                  210                                   215                                   220  
 Ser Leu Glu Glu Phe Ala Ala Phe Val Tyr His Gln Pro Phe Thr Lys  
                                  225                                   230                                   235                                   240  
 Met Ala Tyr Lys Ala His Arg His Leu Leu Asn Phe Asn Gly Tyr Asp  
                                  245                                   250                                   255  
 Thr Asp Lys Asp Ala Ile Glu Gly Ala Leu Gly Gln Thr Thr Ala Tyr  
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 Asn Asn Val Ile Gly Asn Ser Tyr Thr Ala Ser Val Tyr Leu Gly Leu  
                                  275                                   280                                   285  
 Ala Ala Leu Leu Asp Gln Ala Asp Asp Leu Thr Gly Arg Ser Ile Gly  
                                  290                                   295                                   300  
 Phe Leu Ser Tyr Gly Ser Gly Ser Val Ala Glu Phe Phe Ser Gly Thr  
                                  305                                   310                                   315                                   320  
 Val Val Ala Gly Tyr Arg Glu Arg Leu Arg Thr Glu Ala Asn Gln Glu  
                                  325                                   330                                   335  
 Ala Ile Ala Arg Arg Lys Ser Val Asp Tyr Ala Thr Tyr Arg Glu Leu  
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 His Glu Tyr Thr Leu Pro Ser Asp Gly Gly Asp His Ala Thr Pro Val  
                                  355                                   360                                   365  
 Gln Thr Thr Gly Pro Phe Arg Leu Ala Gly Ile Asn Asp His Lys Arg  
                                  370                                   375                                   380  
 Ile Tyr Glu Ala Arg  
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 <211> 389  
 <212> PRT  
 <213> Streptomyces griseoliosporeus

&lt;400&gt; 83

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Met Pro Leu Ala Ile Gly Ile His Asp Leu Ser Phe Ala Thr Gly Glu
1      5      10      15
Phe Val Leu Pro His Thr Ala Leu Ala Ala His Asn Gly Thr Glu Ile
20      25      30
Gly Lys Tyr His Ala Gly Ile Gly Gln Glu Ser Met Ser Val Pro Ala
35      40      45
Ala Asp Glu Asp Ile Val Thr Leu Ala Ala Thr Ala Ala Ala Pro Ile
50      55      60
Val Ala Arg His Gly Ser Asp Arg Ile Arg Thr Val Val Leu Ala Thr
65      70      75      80
Glu Ser Ser Ile Asp Gln Ala Lys Ser Ala Gly Val Tyr Val His Ser
85      90      95
Leu Leu Gly Leu Pro Ser Ala Thr Arg Val Val Glu Leu Lys Gln Ala
100     105     110
Cys Tyr Gly Ala Thr Ala Gly Leu Gln Phe Ala Ile Gly Leu Val Gln
115     120     125
Arg Asp Pro Ala Gln Gln Val Leu Val Ile Ala Ser Asp Val Ser Lys
130     135     140
Tyr Asp Leu Asp Ser Pro Gly Glu Ala Thr Gln Gly Ala Ala Ala Val
145     150     155     160
Ala Met Leu Val Gly Ala Asp Pro Gly Leu Val Arg Ile Glu Asp Pro
165     170     175
Ser Gly Leu Phe Thr Val Asp Val Met Asp Phe Trp Arg Pro Asn Tyr
180     185     190
Arg Thr Thr Ala Leu Val Asp Gly Gln Glu Ser Ile Gly Ala Tyr Leu
195     200     205
Gln Ala Val Glu Gly Ala Trp Lys Asp Tyr Ser Glu Arg Gly Gly His
210     215     220
Ser Leu Glu Gln Phe Ala Ala Phe Cys Tyr His Gln Pro Phe Thr Lys
225     230     235     240
Met Ala His Lys Ala His Arg His Leu Leu Asn Tyr Cys Ser His Asp
245     250     255
Ile His His Asp Asp Val Thr Arg Ala Val Gly Arg Thr Thr Ala Tyr
260     265     270
Asn Arg Val Ile Gly Asn Ser Tyr Thr Ala Ser Val Tyr Leu Gly Leu
275     280     285
Ala Ala Leu Leu Asp Gln Ala Asp Asp Leu Thr Gly Glu Arg Ile Gly

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290                      295                      300  
 Phe Leu Ser Tyr Gly Ser Gly Ser Val Ala Glu Phe Phe Gly Gly Ile  
 305                      310                      315                      320  
 Val Val Ala Gly Tyr Arg Asp Arg Leu Arg Thr Ala Ala Asn Ile Glu  
                          325                      330                      335  
 Ala Val Ser Arg Arg Arg Pro Ile Asp Tyr Ala Gly Tyr Asn Glu Leu  
                          340                      345                      350  
 His Glu Trp Ala Phe Pro Ala Arg Arg Gly Ala His Ser Thr Pro Gln  
                          355                      360                      365  
 Gln Thr Thr Gly Pro Phe Arg Leu Ser Gly Ile Ser Gly His Lys Arg  
                          370                      375                      380  
 Leu Tyr Arg Ala Cys  
 385  
 <210> 84  
 <211> 407  
 <212> PRT  
 <213> *Borrelia burgdorferi*  
  
 <400> 84  
 Met Arg Ile Gly Ile Ser Asp Ile Arg Ile Phe Leu Pro Leu Asn Tyr  
 1                      5                      10                      15  
 Leu Asp Phe Ser Val Leu Leu Glu Asn Pro Leu Tyr Phe Ser Asn Glu  
                          20                      25                      30  
 Val Phe Phe Lys Lys Ile Asn Arg Ala Ile Asp Ala Thr Leu Gln Lys  
                          35                      40                      45  
 Gly Phe Arg Phe Thr Ser Pro Asn Glu Asp Ser Val Thr Met Ala Ser  
                          50                      55                      60  
 Ser Ala Val Lys Leu Ile Phe Asp Asn Asn Asn Leu Asp Leu Ser Lys  
 65                      70                      75                      80  
 Ile Arg Ile Leu Leu Gly Gly Thr Glu Thr Gly Val Asp His Ser Lys  
                          85                      90                      95  
 Ala Ile Ser Ser Tyr Val Phe Gly Ala Leu Lys Gln Ser Gly Ile Cys  
                          100                      105                      110  
 Leu Gly Asn Asn Phe Leu Thr Phe Gln Val Gln His Ala Cys Ala Gly  
                          115                      120                      125  
 Ala Ala Met Ser Leu His Thr Val Ala Ser Val Leu Ser His Ser Asn

130	135	140
Asn Ser Glu Tyr Gly Ile Val Phe Ser Ser Asp Ile Ala His Tyr Ser 145 150 155 160		
Asn Leu Thr Thr Ala Glu Ile Thr Gln Gly Ala Gly Ala Thr Ala Ile 165 170 175		
Leu Ile Glu Lys Asn Pro Lys Ile Leu Ser Ile Asn Leu Ser Glu Phe 180 185 190		
Gly Val Tyr Thr Asp Asp Val Asp Asp Phe Phe Arg Pro Phe Gly Ser 195 200 205		
Val Glu Ala Lys Val Arg Gly Gln Tyr Ser Val Glu Cys Tyr Asn Asn 210 215 220		
Ala Asn Glu Asn Ala Leu Arg Asp Phe Ala Phe Lys Lys Gln Leu Ser 225 230 235 240		
Met Lys Asp Leu Phe Ser Asn Tyr Arg Phe Val Leu His Val Pro Phe 245 250 255		
Ala Lys Met Pro Ile Asp Ser Met His Tyr Ile Leu Lys Lys Tyr Tyr 260 265 270		
Ser Asp Asp Glu Ser Val Arg Asn Ala Tyr Leu Glu Ser Ile Asp Phe 275 280 285		
Tyr Asp Gly Val Glu Ala Ala Met Glu Val Gly Asn Leu Tyr Thr Gly 290 295 300		
Ser Ile Phe Leu Ser Leu Ala Phe Tyr Leu Lys Arg Val Phe Ser Lys 305 310 315 320		
Lys Asp Ile Thr Gly Glu Lys Ile Leu Phe Cys Ser Tyr Gly Ser Gly 325 330 335		
Asn Ile Met Ile Ile Tyr Glu Leu Thr Ile Glu Lys Ser Ala Phe Asp 340 345 350		
Val Ile Lys Leu Trp Asp Leu Glu Gly Leu Ile Lys Asn Arg Asn Asn 355 360 365		
Ala Asn Phe Glu Glu Tyr Lys Asp Phe Phe Gln Asn Lys Ile Ile Pro 370 375 380		
Gly Glu Ser Arg Gly Phe Tyr Leu Lys Glu Leu Arg Asn Asp Gly Tyr 385 390 395 400		
Arg Val Tyr Gly Tyr Arg Ala 405		

&lt;210&gt; 85

&lt;211&gt; 317

&lt;212&gt; PRT

<213> *Streptococcus pneumoniae*

&lt;400&gt; 85

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Met Asp Arg Glu Pro Val Thr Val Arg Ser Tyr Ala Asn Ile Ala Ile
1          5          10          15

Ile Lys Tyr Trp Gly Lys Lys Lys Glu Lys Glu Met Val Pro Ala Thr
20          25          30

Ser Ser Ile Ser Leu Thr Leu Glu Asn Met Tyr Thr Glu Thr Thr Leu
35          40          45

Ser Pro Leu Pro Ala Asn Val Thr Ala Asp Glu Phe Tyr Ile Asn Gly
50          55          60

Gln Leu Gln Asn Glu Val Glu His Ala Lys Met Ser Lys Ile Ile Asp
65          70          75          80

Arg Tyr Arg Pro Ala Gly Glu Gly Phe Val Arg Ile Asp Thr Gln Asn
85          90          95

Asn Met Pro Thr Ala Ala Gly Leu Ser Ser Ser Ser Gly Leu Ser
100         105         110

Ala Leu Val Lys Ala Cys Asn Ala Tyr Phe Lys Leu Gly Leu Asp Arg
115         120         125

Ser Gln Leu Ala Gln Glu Ala Lys Phe Ala Ser Gly Ser Ser Ser Arg
130         135         140

Ser Phe Tyr Gly Pro Leu Gly Ala Trp Asp Lys Asp Ser Gly Glu Ile
145         150         155         160

Tyr Pro Val Glu Thr Asp Leu Lys Leu Ala Met Ile Met Leu Val Leu
165         170         175

Glu Asp Lys Lys Lys Pro Ile Ser Ser Arg Asp Gly Met Lys Leu Cys
180         185         190

Val Glu Thr Ser Thr Thr Phe Asp Asp Trp Val Arg Gln Ser Glu Lys
195         200         205

Asp Tyr Gln Asp Met Leu Ile Tyr Leu Lys Glu Asn Asp Phe Ala Lys
210         215         220

Ile Gly Glu Leu Thr Glu Lys Asn Ala Leu Ala Met His Ala Thr Thr
225         230         235         240

Lys Thr Ala Ser Pro Ala Phe Ser Tyr Leu Thr Asp Ala Ser Tyr Glu
245         250         255

Ala Met Ala Phe Val Arg Gln Leu Arg Glu Lys Gly Glu Ala Cys Tyr

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260                      265                      270  
 Phe Thr Met Asp Ala Gly Pro Asn Val Lys Val Phe Cys Gln Glu Lys  
       275                      280                      285  
 Asp Leu Glu His Leu Ser Glu Ile Phe Gly Gln Arg Tyr Arg Leu Ile  
       290                      295                      300  
 Val Ser Lys Thr Lys Asp Leu Ser Gln Asp Asp Cys Cys  
       305                      310                      315  
 <210> 86  
 <211> 314  
 <212> PRT  
 <213> Streptococcus pyrogenes  
  
 <400> 86  
 Met Asp Pro Asn Val Ile Thr Val Thr Ser Tyr Ala Asn Ile Ala Ile  
       1                      5                      10                      15  
 Ile Lys Tyr Trp Gly Lys Glu Asn Gln Ala Lys Met Ile Pro Ser Thr  
       20                      25  
 Ser Ser Ile Ser Leu Thr Leu Glu Asn Met Phe Thr Thr Thr Ser Val  
       35                      40                      45  
 Ser Phe Leu Pro Asp Thr Ala Thr Ser Asp Gln Phe Tyr Ile Asn Gly  
       50                      55                      60  
 Val Leu Gln Asn Asp Glu Glu His Thr Lys Ile Ser Thr Ile Ile Asp  
       65                      70                      75                      80  
 Gln Phe Arg Gln Pro Gly Gln Ala Phe Val Lys Met Glu Thr Gln Asn  
       85                      90                      95  
 Asn Met Pro Thr Ala Ala Gly Leu Ser Ser Ser Ser Ser Gly Leu Ser  
       100                      105                      110  
 Ala Leu Val Lys Ala Cys Asp Gln Leu Phe Asp Thr Gln Leu Asp Gln  
       115                      120                      125  
 Lys Ala Leu Ala Gln Lys Ala Lys Phe Ala Ser Gly Ser Ser Ser Arg  
       130                      135                      140  
 Ser Phe Phe Gly Pro Val Ala Ala Trp Asp Lys Asp Ser Gly Ala Ile  
       145                      150                      155                      160  
 Tyr Lys Val Glu Thr Asp Leu Lys Met Ala Met Ile Met Leu Val Leu  
       165                      170                      175  
 Asn Ala Ala Lys Lys Pro Ile Ser Ser Arg Glu Gly Met Lys Leu Cys



180                      185                      190  
 Arg Asp Thr Ser Thr Thr Phe Asp Glu Trp Val Glu Gln Ser Ala Ile  
     195                      200                      205  
 Asp Tyr Gln His Met Leu Thr Tyr Leu Lys Thr Asn Asn Phe Glu Lys  
     210                      215                      220  
 Val Gly Gln Leu Thr Glu Ala Asn Ala Leu Ala Met His Ala Thr Thr  
     225                      230                      235                      240  
 Lys Thr Ala Asn Pro Pro Phe Ser Tyr Leu Thr Lys Glu Ser Tyr Gln  
                     245                      250                      255  
 Ala Met Glu Ala Val Lys Glu Leu Arg Gln Glu Gly Phe Ala Cys Tyr  
                     260                      265                      270  
 Phe Thr Met Asp Ala Gly Pro Asn Val Lys Val Leu Cys Leu Glu Lys  
     275                      280                      285  
 Asp Leu Ala Gln Leu Ala Glu Arg Leu Gly Lys Asn Tyr Arg Ile Ile  
     290                      295                      300  
 Val Ser Lys Thr Lys Asp Leu Pro Asp Val  
     305                      310  
 <210> 87  
 <211> 331  
 <212> PRT  
 <213> Enterococcus faecalis  
  
 <400> 87  
 Met Leu Ser Gly Lys Ala Arg Ala His Thr Asn Ile Ala Leu Ile Lys  
     1                      5                      10                      15  
 Tyr Trp Gly Lys Ala Asn Glu Glu Tyr Ile Leu Pro Met Asn Ser Ser  
                     20                      25                      30  
 Leu Ser Leu Thr Leu Asp Ala Phe Tyr Thr Glu Thr Thr Val Thr Phe  
     35                      40                      45  
 Asp Ala His Tyr Ser Glu Asp Val Phe Ile Leu Asn Gly Ile Leu Gln  
     50                      55                      60  
 Asn Glu Lys Gln Thr Lys Lys Val Lys Glu Phe Leu Asn Leu Val Arg  
     65                      70                      75                      80  
 Gln Gln Ala Asp Cys Thr Trp Phe Ala Lys Val Glu Ser Gln Asn Phe  
                     85                      90                      95  
 Val Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Gly Leu Ala Ala

100                      105                      110  
 Leu Ala Gly Ala Cys Asn Val Ala Leu Gly Leu Asn Leu Ser Ala Lys  
       115                      120                      125  
 Asp Leu Ser Arg Leu Ala Arg Arg Gly Ser Gly Ser Ala Cys Arg Ser  
       130                      135                      140  
 Ile Phe Gly Gly Phe Ala Gln Trp Asn Lys Gly His Ser Asp Glu Thr  
       145                      150                      155                      160  
 Ser Phe Ala Glu Asn Ile Pro Ala Asn Asn Trp Glu Asn Glu Leu Ala  
       165                      170                      175  
 Met Leu Phe Ile Leu Ile Asn Asp Gly Glu Lys Asp Val Ser Ser Arg  
       180                      185                      190  
 Asp Gly Met Lys Arg Thr Val Glu Thr Ser Ser Phe Tyr Gln Gly Trp  
       195                      200                      205  
 Leu Asp Asn Val Glu Lys Asp Leu Ser Gln Val His Glu Ala Ile Lys  
       210                      215                      220  
 Thr Lys Asp Phe Pro Arg Leu Gly Glu Ile Ile Glu Ala Asn Gly Leu  
       225                      230                      235                      240  
 Arg Met His Gly Thr Thr Leu Gly Ala Val Pro Pro Phe Thr Tyr Trp  
       245                      250                      255  
 Ser Pro Gly Ser Leu Gln Ala Met Ala Leu Val Arg Gln Ala Arg Ala  
       260                      265                      270  
 Lys Gly Ile Pro Cys Tyr Phe Thr Met Asp Ala Gly Pro Asn Val Lys  
       275                      280                      285  
 Val Leu Val Glu Lys Lys Asn Leu Glu Ala Leu Lys Thr Phe Leu Ser  
       290                      295                      300  
 Glu His Phe Ser Lys Glu Gln Leu Val Pro Ala Phe Ala Gly Pro Gly  
       305                      310                      315                      320  
 Ile Glu Leu Phe Glu Thr Lys Gly Met Asp Lys  
       325                      330  
 <210> 88  
 <211> 325  
 <212> PRT  
 <213> Enterococcus faecium  
  
 <400> 88  
 Met Phe Lys Gly Lys Ala Arg Ala Tyr Thr Asn Ile Ala Leu Ile Lys

1	5	10	15
Tyr Trp Gly	Lys Lys Asn Glu Glu	Leu Ile Leu Pro Met	Asn Asn Ser
	20	25	30
Leu Ser Leu Thr	Leu Asp Ala Phe Tyr Thr	Glu Thr Glu Val Ile Phe	
	35	40	45
Ser Asp Ser Tyr Met Val	Asp Glu Phe Tyr Leu Asp	Gly Thr Leu Gln	
	50	55	60
Asp Glu Lys Ala Thr Lys	Lys Val Ser Gln Phe Leu Asp	Leu Phe Arg	
	65	70	75
Lys Glu Ala Gly Leu Ser	Leu Lys Ala Ser Val Ile Ser	Gln Asn Phe	
	85	90	95
Val Pro Thr Ala Ala Gly	Leu Ala Ser Ser Ala Ser	Gly Leu Ala Ala	
	100	105	110
Leu Ala Gly Ala Cys Asn Thr	Ala Leu Lys Leu Gly Leu Asp	Asp Leu	
	115	120	125
Ser Leu Ser Arg Phe Ala Arg	Arg Gly Ser Gly Ser Ala Cys Arg	Ser	
	130	135	140
Ile Phe Gly Gly Phe Val Glu Trp	Glu Lys Gly His Asp Asp	Leu Ser	
	145	150	155
Ser Tyr Ala Lys Pro Val Pro	Ser Asp Ser Phe Glu Asp Asp	Leu Ala	
	165	170	175
Met Val Phe Val Leu Ile Asn Asp	Gln Lys Lys Glu Val Ser	Ser Arg	
	180	185	190
Asn Gly Met Arg Arg Thr Val	Glu Thr Ser Asn Phe Tyr Gln Gly Trp		
	195	200	205
Leu Asp Ser Val Glu Gly Asp	Leu Tyr Gln Leu Lys Gln Ala Ile Lys		
	210	215	220
Thr Lys Asp Phe Gln Leu Leu Gly	Glu Thr Met Glu Arg Asn Gly Leu		
	225	230	235
Lys Met His Gly Thr Thr Leu Ala Ala	Gln Pro Pro Phe Thr Tyr Trp		
	245	250	255
Ser Pro Asn Ser Ser Leu Lys Ala Met	Asp Ala Val Arg Gln Leu Arg Lys		
	260	265	270
Gln Gly Ile Pro Cys Tyr Phe Thr	Met Asp Ala Gly Pro Asn Val Lys		
	275	280	285
Val Leu Val Glu Asn Ser His Leu Ser	Glu Val Gln Glu Thr Phe Thr		
	290	295	300
Lys Leu Phe Ser Lys Glu Gln Val Ile Thr	Ala His Ala Gly Pro Gly		

305                      310                      315                      320  
 Ile Ala Ile Ile Glu  
                          325  
 <210> 89  
 <211> 327  
 <212> PRT  
 <213> Staphylococcus haemolyticus  
  
 <400> 89  
 Met Lys Lys Ser Gly Lys Ala Arg Ala His Thr Asn Ile Ala Leu Ile  
 1                      5                      10                      15  
 Lys Tyr Trp Gly Lys Ala Asp Glu Ala Leu Ile Ile Pro Met Asn Asn  
                          20                      25                      30  
 Ser Leu Ser Val Thr Leu Asp Arg Phe Tyr Thr Glu Thr Arg Val Thr  
                          35                      40                      45  
 Phe Asp Glu Thr Leu Thr Glu Asp Gln Leu Ile Leu Asn Gly Glu Ala  
                          50                      55                      60  
 Val Asn Ala Lys Glu Ser Ala Lys Ile Gln Arg Tyr Met Glu Met Ile  
 65                      70                      75                      80  
 Arg Lys Glu Ala Gly Ile Ser His Glu Ala Leu Ile Glu Ser Glu Asn  
                          85                      90                      95  
 Phe Val Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Ala Tyr Ala  
                          100                      105                      110  
 Ala Leu Ala Gly Ala Cys Asn Glu Ala Leu Gln Leu Gly Leu Ser Asp  
                          115                      120                      125  
 Lys Asp Leu Ser Arg Leu Ala Arg Arg Gly Ser Gly Ser Ala Ser Arg  
                          130                      135                      140  
 Ser Ile Tyr Gly Gly Phe Ala Glu Trp Glu Lys Gly Asn Asp Asp Glu  
 145                      150                      155                      160  
 Thr Ser Phe Ala His Arg Val Glu Ala Asp Gly Trp Glu Asn Glu Leu  
                          165                      170                      175  
 Ala Met Val Phe Val Val Ile Asn Asn Lys Ser Lys Lys Val Ser Ser  
                          180                      185                      190  
 Arg Ser Gly Met Ser Leu Thr Arg Asp Thr Ser Arg Phe Tyr Gln Tyr  
                          195                      200                      205  
 Trp Leu Asp Asn Val Glu Pro Asp Leu Lys Glu Thr Lys Glu Ala Ile

210                      215                      220  
 Ala Gln Lys Asp Phe Lys Arg Met Gly Glu Val Ile Glu Ala Asn Gly  
 225                      230                      235                      240  
 Leu Arg Met His Ala Thr Asn Leu Gly Ala Gln Pro Pro Phe Thr Tyr  
                     245                      250                      255  
 Leu Val Pro Glu Ser Tyr Asp Ala Met Arg Ile Val His Glu Cys Arg  
                     260                      265                      270  
 Glu Ala Gly Leu Pro Cys Tyr Phe Thr Met Asp Ala Gly Pro Asn Val  
                     275                      280                      285  
 Lys Val Leu Ile Glu Lys Lys Asn Gln Gln Ala Ile Val Asp Lys Phe  
                     290                      295                      300  
 Leu Gln Glu Phe Asp Gln Ser Gln Ile Ile Thr Ser Asp Ile Thr Gln  
 305                      310                      315                      320  
 Ser Gly Val Glu Ile Ile Lys  
                     325  
  
 <210> 90  
 <211> 327  
 <212> PRT  
 <213> Staphylococcus epidermis  
  
 <400> 90  
 Met Val Lys Ser Gly Lys Ala Arg Ala His Thr Asn Ile Ala Leu Ile  
 1                      5                      10                      15  
 Lys Tyr Trp Gly Lys Ala Asp Glu Thr Tyr Ile Ile Pro Met Asn Asn  
                     20                      25                      30  
 Ser Leu Ser Val Thr Leu Asp Arg Phe Tyr Thr Glu Thr Lys Val Thr  
                     35                      40                      45  
 Phe Asp Pro Asp Phe Thr Glu Asp Cys Leu Ile Leu Asn Gly Asn Glu  
                     50                      55                      60  
 Val Asn Ala Lys Glu Lys Glu Lys Ile Gln Asn Tyr Met Asn Ile Val  
 65                      70                      75                      80  
 Arg Asp Leu Ala Gly Asn Arg Leu His Ala Arg Ile Glu Ser Glu Asn  
                     85                      90                      95  
 Tyr Val Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Ala Tyr Ala  
                     100                      105                      110  
 Ala Leu Ala Ala Ala Cys Asn Glu Ala Leu Ser Leu Asn Leu Ser Asp

115                      120                      125  
 Thr Asp Leu Ser Arg Leu Ala Arg Arg Gly Ser Gly Ser Ala Ser Arg  
 130                      135                      140  
 Ser Ile Phe Gly Gly Phe Ala Glu Trp Glu Lys Gly His Asp Asp Leu  
 145                      150                      155                      160  
 Thr Ser Tyr Ala His Gly Ile Asn Ser Asn Gly Trp Glu Lys Asp Leu  
 165                      170                      175  
 Ser Met Ile Phe Val Val Ile Asn Asn Gln Ser Lys Lys Val Ser Ser  
 180                      185                      190  
 Arg Ser Gly Met Ser Leu Thr Arg Asp Thr Ser Arg Phe Tyr Gln Tyr  
 195                      200                      205  
 Trp Leu Asp His Val Asp Glu Asp Leu Asn Glu Ala Lys Glu Ala Val  
 210                      215                      220  
 Lys Asn Gln Asp Phe Gln Arg Leu Gly Glu Val Ile Glu Ala Asn Gly  
 225                      230                      235                      240  
 Leu Arg Met His Ala Thr Asn Leu Gly Ala Gln Pro Pro Phe Thr Tyr  
 245                      250                      255  
 Leu Val Gln Glu Ser Tyr Asp Ala Met Ala Ile Val Glu Gln Cys Arg  
 260                      265                      270  
 Lys Ala Asn Leu Pro Cys Tyr Phe Thr Met Asp Ala Gly Pro Asn Val  
 275                      280                      285  
 Lys Val Leu Val Glu Lys Lys Asn Lys Gln Ala Val Met Glu Gln Phe  
 290                      295                      300  
 Leu Lys Val Phe Asp Glu Ser Lys Ile Ile Ala Ser Asp Ile Ile Ser  
 305                      310                      315                      320  
 Ser Gly Val Glu Ile Ile Lys  
 325

&lt;210&gt; 91

&lt;211&gt; 327

&lt;212&gt; PRT

&lt;213&gt; Staphylococcus aureus

&lt;400&gt; 91

Met Ile Lys Ser Gly Lys Ala Arg Ala His Thr Asn Ile Ala Leu Ile  
 1                      5                      10                      15  
 Lys Tyr Trp Gly Lys Lys Asp Glu Ala Leu Ile Ile Pro Met Asn Asn

	20		25		30	
Ser Ile Ser Val Thr Leu Glu Lys Phe Tyr Thr Glu Thr Lys Val Thr	35		40		45	
Phe Asn Asp Gln Leu Thr Gln Asp Gln Phe Trp Leu Asn Gly Glu Lys	50		55		60	
Val Ser Gly Lys Glu Leu Glu Lys Ile Ser Lys Tyr Met Asp Ile Val	65	70		75		80
Arg Asn Arg Ala Gly Ile Asp Trp Tyr Ala Glu Ile Glu Ser Asp Asn		85		90		95
Phe Val Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Ala Tyr Ala		100		105		110
Ala Leu Ala Ala Cys Asn Gln Ala Leu Asp Leu Gln Leu Ser Asp		115		120		125
Lys Asp Leu Ser Arg Leu Ala Arg Ile Gly Ser Gly Ser Ala Ser Arg		130		135		140
Ser Ile Tyr Gly Gly Phe Ala Glu Trp Glu Lys Gly Tyr Asn Asp Glu		145	150		155	160
Thr Ser Tyr Ala Val Pro Leu Glu Ser Asn His Phe Glu Asp Asp Leu		165		170		175
Ala Met Ile Phe Val Val Ile Asn Gln His Ser Lys Lys Val Pro Ser		180		185		190
Arg Tyr Gly Met Ser Leu Thr Arg Asn Thr Ser Arg Phe Tyr Gln Tyr		195		200		205
Trp Leu Asp His Ile Asp Glu Asp Leu Ala Glu Ala Lys Ala Ala Ile		210		215		220
Gln Asp Lys Asp Phe Lys Arg Leu Gly Glu Val Ile Glu Glu Asn Gly		225	230		235	240
Leu Arg Met His Ala Thr Asn Leu Gly Ser Thr Pro Pro Phe Thr Tyr		245		250		255
Leu Val Gln Glu Ser Tyr Asp Val Met Ala Leu Val His Glu Cys Arg		260		265		270
Glu Ala Gly Tyr Pro Cys Tyr Phe Thr Met Asp Ala Gly Pro Asn Val		275		280		285
Lys Ile Leu Val Glu Lys Lys Asn Lys Gln Gln Ile Ile Asp Lys Leu		290		295		300
Leu Thr Gln Phe Asp Asn Asn Gln Ile Ile Asp Ser Asp Ile Ile Ala		305	310		315	320
Thr Gly Ile Glu Ile Ile Glu						

325

&lt;210&gt; 92

&lt;211&gt; 350

&lt;212&gt; PRT

&lt;213&gt; Streptomyces sp. CL190

&lt;400&gt; 92

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Met Arg Ser Glu His Pro Thr Thr Thr Val Leu Gln Ser Arg Glu Gln
 1           5           10           15

Gly Ser Ala Ala Gly Ala Thr Ala Val Ala His Pro Asn Ile Ala Leu
 20           25           30

Ile Lys Tyr Trp Gly Lys Arg Asp Glu Arg Leu Ile Leu Pro Cys Thr
 35           40           45

Thr Ser Leu Ser Met Thr Leu Asp Val Phe Pro Thr Thr Thr Glu Val
 50           55           60

Arg Leu Asp Pro Ala Ala Glu His Asp Thr Ala Ala Leu Asn Gly Glu
 65           70           75           80

Val Ala Thr Gly Glu Thr Leu Arg Arg Ile Ser Ala Phe Leu Ser Leu
 85           90           95

Val Arg Glu Val Ala Gly Ser Asp Gln Arg Ala Val Val Asp Thr Arg
 100          105          110

Asn Thr Val Pro Thr Gly Ala Gly Leu Ala Ser Ser Ala Ser Gly Phe
 115          120          125

Ala Ala Leu Ala Val Ala Ala Ala Ala Tyr Gly Leu Glu Leu Asp
 130          135          140

Asp Arg Gly Leu Ser Arg Leu Ala Arg Arg Gly Ser Gly Ser Ala Ser
 145          150          155          160

Arg Ser Ile Phe Gly Gly Phe Ala Val Trp His Ala Gly Pro Asp Gly
 165          170          175

Thr Ala Thr Glu Ala Asp Leu Gly Ser Tyr Ala Glu Pro Val Pro Ala
 180          185          190

Ala Asp Leu Asp Pro Ala Leu Val Ile Ala Val Val Asn Ala Gly Pro
 195          200          205

Lys Pro Val Ser Ser Arg Glu Ala Met Arg Arg Thr Val Asp Thr Ser
 210          215          220

Pro Leu Tyr Arg Pro Trp Ala Asp Ser Ser Lys Asp Asp Leu Asp Glu

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225                      230                      235                      240  
 Met Arg Ser Ala Leu Leu Arg Gly Asp Leu Glu Ala Val Gly Glu Ile  
                                  245                      250                      255  
 Ala Glu Arg Asn Ala Leu Gly Met His Ala Thr Met Leu Ala Ala Arg  
                                  260                      265                      270  
 Pro Ala Val Arg Tyr Leu Ser Pro Ala Thr Val Thr Val Leu Asp Ser  
                                  275                      280                      285  
 Val Leu Gln Leu Arg Lys Asp Gly Val Leu Ala Tyr Ala Thr Met Asp  
                                  290                      295                      300  
 Ala Gly Pro Asn Val Lys Val Leu Cys Arg Arg Ala Asp Ala Glu Arg  
                                  305                      310                      315                      320  
 Val Ala Asp Val Val Arg Ala Ala Ala Ser Gly Gly Gln Val Leu Val  
                                  325                      330                      335  
 Ala Gly Pro Gly Asp Gly Ala Arg Leu Leu Ser Glu Gly Ala  
                                  340                      345                      350  
  
 <210> 93  
 <211> 331  
 <212> PRT  
 <213> Streptomyces griseolosporeus  
  
 <400> 93  
 Ala Thr Ala Val Ala Gln Pro Asn Ile Ala Leu Ile Lys Tyr Trp Gly  
   1                      5                      10                      15  
 Lys Lys Asp Glu His Leu Val Leu Pro Arg Thr Asp Ser Leu Ser Met  
                                  20                      25                      30  
 Thr Leu Asp Ile Phe Pro Thr Thr Thr Arg Val Gln Leu Ala Pro Gly  
                                  35                      40                      45  
 Ala Gly Gln Asp Thr Val Ala Phe Asn Gly Glu Pro Ala Thr Gly Glu  
                                  50                      55                      60  
 Ala Glu Arg Arg Ile Thr Ala Phe Leu Arg Leu Val Arg Glu Arg Ser  
                                  65                      70                      75                      80  
 Gly Arg Thr Glu Arg Ala Arg Val Glu Thr Glu Asn Thr Val Pro Thr  
                                  85                      90                      95  
 Gly Ala Gly Leu Ala Ser Ser Ala Ser Gly Phe Ala Ala Leu Ala Val  
                                  100                      105                      110  
 Ala Ala Ala Ala Ala Tyr Gly Leu Gly Leu Asp Ala Arg Gly Leu Ser

115                      120                      125  
 Arg Leu Ala Arg Arg Gly Ser Gly Ser Ala Ser Arg Ser Ile Phe Asp  
 130                      135                      140  
 Gly Phe Ala Val Trp His Ala Gly His Ala Gly Gly Thr Pro Glu Glu  
 145                      150                      155                      160  
 Ala Asp Leu Gly Ser Tyr Ala Glu Pro Val Pro Ala Val Asp Leu Glu  
 165                      170                      175  
 Pro Ala Leu Val Val Ala Val Val Ser Ala Ala Pro Lys Ala Val Ser  
 180                      185                      190  
 Ser Arg Glu Ala Met Arg Arg Thr Val Asp Thr Ser Pro Leu Tyr Glu  
 195                      200                      205  
 Pro Trp Ala Val Ser Ser Arg Ala Asp Leu Ala Asp Ile Gly Ala Ala  
 210                      215                      220  
 Leu Ala Arg Gly Asn Leu Pro Ala Val Gly Glu Ile Ala Glu Arg Asn  
 225                      230                      235                      240  
 Ala Leu Gly Met His Ala Thr Met Leu Ala Ala Arg Pro Ala Val Arg  
 245                      250                      255  
 Tyr Leu Ser Pro Ala Ser Leu Ala Val Leu Asp Gly Val Leu Gln Leu  
 260                      265                      270  
 Arg Arg Asp Gly Val Pro Ala Tyr Ala Thr Met Asp Ala Gly Pro Asn  
 275                      280                      285  
 Val Lys Val Leu Cys Pro Arg Ser Asp Ala Glu Arg Val Ala Glu Ala  
 290                      295                      300  
 Leu Arg Ala Ala Ala Pro Val Gly Ala Val His Ile Ala Gly Pro Gly  
 305                      310                      315                      320  
 Arg Gly Ala Arg Leu Val Ala Glu Glu Cys Arg  
 325                      330

&lt;210&gt; 94

&lt;211&gt; 312

&lt;212&gt; PRT

<213> *Borrelia burgdorferi*

&lt;400&gt; 94

Met Lys Ile Lys Cys Lys Val His Ala Ser Leu Ala Leu Ile Lys Tyr  
 1                      5                      10                      15  
 Trp Gly Lys Lys Asp Val Phe Leu Asn Ile Pro Ala Thr Ser Ser Leu

20					25					30						
Ala	Val	Ser	Val	Asp	Lys	Phe	Tyr	Ser	Ile	Ser	Glu	Leu	Glu	Leu	Ser	
35					40					45						
Asn	Arg	Asp	Glu	Ile	Ile	Leu	Asn	Ser	Lys	Pro	Val	Ile	Leu	Lys	Asn	
50					55					60						
Arg	Glu	Lys	Val	Phe	Phe	Asp	Tyr	Ala	Arg	Lys	Ile	Leu	Asn	Glu	Pro	
65					70					75					80	
Asn	Val	Arg	Phe	Lys	Ile	Lys	Ser	Lys	Asn	Asn	Phe	Pro	Thr	Ala	Ala	
85					90					95						
Gly	Leu	Ala	Ser	Ser	Ser	Ser	Gly	Phe	Ala	Ser	Ile	Ala	Ala	Cys	Ile	
100					105					110						
Leu	Lys	Tyr	Phe	Asn	Lys	Tyr	Ser	Cys	Asn	Ser	Ala	Ser	Asn	Leu	Ala	
115					120					125						
Arg	Val	Gly	Ser	Ala	Ser	Ala	Ala	Arg	Ala	Ile	Tyr	Gly	Gly	Phe	Thr	
130					135					140						
Ile	Leu	Lys	Glu	Gly	Ser	Lys	Glu	Ser	Phe	Gln	Leu	Arg	Asp	Gln	Ser	
145					150					155					160	
Tyr	Phe	Asn	Asp	Leu	Arg	Ile	Ile	Phe	Ala	Ile	Ile	Asp	Ser	Asn	Glu	
165					170					175						
Lys	Glu	Leu	Ser	Ser	Arg	Ala	Ala	Met	Asn	Ile	Cys	Lys	Arg	His	Lys	
180					185					190						
Phe	Tyr	Tyr	Asp	Ala	Trp	Ile	Ala	Ser	Ser	Lys	Lys	Ile	Phe	Lys	Asp	
195					200					205						
Ala	Leu	Tyr	Phe	Phe	Leu	Lys	Lys	Asp	Phe	Ile	His	Phe	Gly	Ala	Thr	
210					215					220						
Ile	Val	Lys	Ser	Tyr	Gln	Asn	Met	Phe	Ala	Leu	Met	Phe	Ala	Ser	Ser	
225					230					235					240	
Ile	Phe	Tyr	Phe	Lys	Asn	Ser	Thr	Ile	Asp	Leu	Ile	Arg	Tyr	Ala	Ala	
245					250					255						
Asp	Leu	Arg	Asn	Glu	Gly	Ile	Phe	Val	Phe	Glu	Thr	Met	Asp	Ala	Gly	
260					265					270						
Pro	Gln	Val	Lys	Phe	Leu	Cys	Leu	Glu	Glu	Asn	Leu	Asn	Thr	Ile	Leu	
275					280					285						
Lys	Gly	Leu	Lys	Gln	Asn	Phe	Thr	Gly	Ile	Asp	Phe	Ile	Val	Ser	Lys	
290					295					300						
Val	Gly	Cys	Asp	Leu	Glu	Trp	Ile									
305					310											

&lt;210&gt; 95

&lt;211&gt; 292

&lt;212&gt; PRT

&lt;213&gt; Streptococcus pneumoniae

&lt;400&gt; 95

```

Met Thr Lys Lys Val Gly Val Gly Gln Ala His Ser Lys Ile Ile Leu
 1           5              10              15

Ile Gly Glu His Ala Val Val Tyr Gly Tyr Pro Ala Ile Ser Leu Pro
 20          25              30

Leu Leu Glu Val Glu Val Thr Cys Lys Val Val Ser Ala Glu Ser Pro
 35          40              45

Trp Arg Leu Tyr Glu Glu Asp Thr Leu Ser Met Ala Val Tyr Ala Ser
 50          55              60

Leu Glu Tyr Leu Asp Ile Thr Glu Ala Cys Val Arg Cys Glu Ile Asp
 65          70              75              80

Ser Ala Ile Pro Glu Lys Arg Gly Met Gly Ser Ser Ala Ala Ile Ser
 85          90              95

Ile Ala Ala Ile Arg Ala Val Phe Asp Tyr Tyr Gln Ala Asp Leu Pro
100         105              110

His Asp Val Leu Glu Ile Leu Val Asn Arg Ala Glu Met Ile Ala His
115         120              125

Met Asn Pro Ser Gly Leu Asp Ala Lys Thr Cys Leu Ser Asp Gln Pro
130         135              140

Ile Arg Phe Ile Lys Asn Val Gly Phe Thr Glu Leu Glu Met Asp Leu
145         150              155              160

Ser Ala Tyr Leu Val Ile Ala Asp Thr Gly Val Tyr Gly His Thr Arg
165         170              175

Glu Ala Ile Gln Val Val Gln Asn Lys Gly Lys Asp Ala Leu Pro Phe
180         185              190

Leu His Ala Leu Gly Glu Leu Thr Gln Gln Ala Glu Val Ala Ile Ser
195         200              205

Gln Lys Tyr Ala Glu Gly Leu Gly Leu Ile Phe Ser Gln Ala His Leu
210         215              220

His Leu Lys Glu Ile Gly Val Ser Ser Pro Glu Ala Asp Phe Leu Val
225         230              235              240

Glu Thr Ala Leu Ser Tyr Gly Ala Leu Gly Ala Lys Met Ser Gly Gly

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[illegible]

180                      185                      190  
 Leu Ala Lys Leu Gly Ala Leu Thr Gln Ala Leu Glu Arg Ala Ile Asn  
           195                      200                      205  
 Gln Lys Asn Lys Val Ala Ile Gly Gln Leu Met Thr Gln Ala His Ser  
           210                      215                      220  
 Ala Leu Lys Ala Ile Gly Val Ser Ile Ser Lys Ala Asp Gln Leu Val  
           225                      230                      235                      240  
 Glu Ala Ala Leu Arg Ala Gly Ala Leu Gly Ala Lys Met Thr Gly Gly  
           245                      250                      255  
 Gly Leu Gly Gly Cys Met Ile Ala Leu Ala Asp Thr Lys Asp Met Ala  
           260                      265                      270  
 Glu Lys Ile Ser His Lys Leu Lys Glu Glu Gly Ala Val Asn Thr Trp  
           275                      280                      285  
 Ile Gln Met Leu  
           290  
 <210> 97  
 <211> 314  
 <212> PRT  
 <213> Enterococcus faecalis  
  
 <400> 97  
 Met Asn Ile Lys Lys Gln Gly Leu Gly Gln Ala Thr Gly Lys Ile Ile  
   1                      5                      10                      15  
 Leu Met Gly Glu His Ala Val Val Tyr Gly Glu Pro Ala Ile Ala Phe  
           20                      25                      30  
 Pro Phe Gln Ala Thr Glu Ile Thr Ala Val Phe Thr Pro Ala Lys Thr  
           35                      40                      45  
 Met Gln Ile Asp Cys Ala Tyr Phe Thr Gly Leu Leu Glu Asp Val Pro  
           50                      55                      60  
 Gln Glu Leu Ala Asn Ile Lys Glu Val Val Gln Gln Thr Leu His Phe  
           65                      70                      75                      80  
 Leu Lys Glu Asp Thr Phe Lys Gly Thr Leu Thr Leu Thr Ser Thr Ile  
           85                      90                      95  
 Pro Ala Glu Arg Gly Met Gly Ser Ser Ala Ala Thr Ala Val Ala Ile  
           100                      105                      110  
 Val Arg Ser Leu Phe Asp Tyr Phe Asp Tyr Ala Tyr Thr Tyr Gln Glu

115                      120                      125  
 Leu Phe Glu Leu Val Ser Leu Ser Glu Lys Ile Ala His Gly Asn Pro  
 130                      135                      140  
 Ser Gly Ile Asp Ala Ala Ala Thr Ser Gly Ala Asp Pro Leu Phe Phe  
 145                      150                      155                      160  
 Thr Arg Gly Phe Pro Pro Thr His Phe Ser Met Asn Leu Ser Asn Ala  
 165                      170  
 Tyr Leu Val Val Ala Asp Thr Gly Ile Lys Gly Gln Thr Arg Glu Ala  
 180                      185                      190  
 Val Lys Asp Ile Ala Gln Leu Ala Gln Asn Asn Pro Thr Ala Ile Ala  
 195                      200                      205  
 Glu Thr Met Lys Gln Leu Gly Ser Phe Thr Lys Glu Ala Lys Gln Ala  
 210                      215                      220  
 Ile Leu Gln Asp Asp Lys Gln Lys Leu Gly Gln Leu Met Thr Leu Ala  
 225                      230                      235                      240  
 Gln Glu Gln Leu Gln Gln Leu Thr Val Ser Asn Asp Met Leu Asp Arg  
 245                      250                      255  
 Leu Val Ala Leu Ser Leu Glu His Gly Ala Leu Gly Ala Lys Leu Thr  
 260                      265                      270  
 Gly Gly Gly Arg Gly Gly Cys Met Ile Ala Leu Thr Asp Asn Lys Lys  
 275                      280                      285  
 Thr Ala Gln Thr Ile Ala Gln Thr Leu Glu Glu Asn Gly Ala Val Ala  
 290                      295                      300  
 Thr Trp Ile Gln Ser Leu Glu Val Lys Lys  
 305                      310  
  
 <210> 98  
 <211> 314  
 <212> PRT  
 <213> Enterococcus faecium  
  
 <400> 98  
 Met Ala Asn Tyr Gly Gln Gly Glu Ser Ser Gly Lys Ile Ile Leu Met  
 1                      5                      10                      15  
 Gly Glu His Ala Val Val Tyr Gly Glu Pro Ala Ile Ala Phe Pro Phe  
 20                      25                      30  
 Tyr Ala Thr Lys Val Thr Ala Phe Leu Glu Glu Leu Asp Ala Met Asp

35					40					45					
Asp	Gln	Leu	Val	Ser	Ser	Tyr	Tyr	Ser	Gly	Asn	Leu	Ala	Glu	Ala	Pro
50						55					60				
His	Ala	Leu	Lys	Asn	Ile	Lys	Lys	Leu	Phe	Ile	His	Leu	Lys	Lys	Gln
65				70					75					80	
His	Asp	Ile	Gln	Lys	Asn	Leu	Gln	Leu	Thr	Ile	Glu	Ser	Thr	Ile	Pro
				85					90					95	
Ala	Glu	Arg	Gly	Met	Gly	Ser	Ser	Ala	Ala	Val	Ala	Thr	Ala	Val	Thr
			100					105					110		
Arg	Ala	Phe	Tyr	Asp	Tyr	Leu	Ala	Phe	Pro	Leu	Ser	Arg	Glu	Ile	Leu
	115					120					125				
Leu	Glu	Asn	Val	Gln	Leu	Ser	Glu	Lys	Ile	Ala	His	Gly	Asn	Pro	Ser
	130			135							140				
Gly	Ile	Asp	Ala	Ala	Ala	Thr	Ser	Ser	Leu	Gln	Pro	Ile	Tyr	Phe	Thr
	145			150					155					160	
Lys	Gly	His	Pro	Phe	Asp	Tyr	Phe	Ser	Leu	Asn	Ile	Asp	Ala	Phe	Leu
			165					170						175	
Ile	Val	Ala	Asp	Thr	Gly	Ile	Lys	Gly	Gln	Thr	Arg	Glu	Ala	Val	Lys
			180					185					190		
Asp	Val	Ala	His	Leu	Phe	Glu	Thr	Gln	Pro	His	Glu	Thr	Gly	Gln	Met
	195					200					205				
Ile	Gln	Lys	Leu	Gly	Tyr	Leu	Thr	Lys	Gln	Ala	Lys	Gln	Ala	Ile	Ile
	210			215							220				
Glu	Asn	Ser	Pro	Glu	Thr	Leu	Ala	Gln	Thr	Met	Asp	Glu	Ser	Gln	Ser
	225			230					235					240	
Leu	Leu	Glu	Lys	Leu	Thr	Ile	Ser	Asn	Asp	Phe	Leu	Asn	Leu	Leu	Ile
			245					250					255		
Gln	Thr	Ala	Lys	Asp	Thr	Gly	Ala	Leu	Gly	Ala	Lys	Leu	Thr	Gly	Gly
			260					265					270		
Gly	Arg	Gly	Gly	Cys	Met	Ile	Ala	Leu	Ala	Gln	Thr	Lys	Thr	Lys	Ala
	275					280					285				
Gln	Glu	Ile	Ser	Gln	Ala	Leu	Glu	Asp	Ala	Gly	Ala	Ala	Glu	Thr	Trp
	290			295							300				
Ile	Gln	Gly	Leu	Gly	Val	His	Thr	Tyr	Val						
	305			310											

&lt;210&gt; 99

&lt;211&gt; 307



&lt;212&gt; PRT

<213> *Staphylococcus haemolyticus*

&lt;400&gt; 99

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Met Val Gln Arg Gly Tyr Gly Glu Ser Asn Gly Lys Ile Ile Leu Ile
 1              5              10              15

Gly Glu His Ala Val Thr Phe Gly Glu Pro Ala Ile Ala Ile Pro Phe
          20              25              30

Thr Ser Gly Lys Val Lys Val Leu Ile Glu Ser Leu Glu Lys Gly Asn
      35              40              45

Tyr Ser Ala Ile Gln Ser Asp Val Tyr Asp Gly Pro Leu Tyr Asp Ala
      50              55              60

Pro Glu His Leu Lys Ser Leu Ile Gly His Phe Val Glu Asn Lys Lys
65              70              75              80

Val Glu Glu Pro Leu Leu Ile Lys Ile Gln Ala Asn Leu Pro Pro Ser
          85              90              95

Arg Gly Leu Gly Ser Ser Ala Ala Val Ala Val Ala Phe Ile Arg Ala
      100              105              110

Ser Tyr Asp Tyr Leu Gly Leu Pro Leu Thr Asp Lys Glu Leu Leu Glu
      115              120              125

Asn Ala Asp Trp Ala Glu Arg Ile Ala His Gly Lys Pro Ser Gly Ile
      130              135              140

Asp Thr Lys Thr Ile Val Thr Asn Gln Pro Val Trp Tyr Gln Lys Gly
145              150              155              160

Glu Val Glu Ile Leu Lys Thr Leu Asp Leu Asp Gly Tyr Met Val Val
          165              170              175

Ile Asp Thr Gly Val Lys Gly Ser Thr Lys Gln Ala Val Glu Asp Val
      180              185              190

His Gln Leu Cys Asp Asn Asp Lys Asn Tyr Met Gln Val Val Lys His
      195              200              205

Ile Gly Ser Leu Val Tyr Ser Ala Ser Glu Ala Ile Glu His His Ser
      210              215              220

Phe Asp Gln Leu Ala Thr Ile Phe Asn Gln Cys Gln Asp Asp Leu Arg
225              230              235              240

Thr Leu Thr Val Ser His Asp Lys Ile Glu Met Phe Leu Arg Leu Gly
          245              250              255

Glu Glu Asn Gly Ser Val Ala Gly Lys Leu Thr Gly Gly Gly Arg Gly

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260265270

Gly Ser Met Leu Ile Leu Ala Lys Glu Leu Gln Thr Ala Lys Asn Ile  
          275280285

Val Ala Ala Val Glu Lys Ala Gly Ala Gln His Thr Trp Ile Glu Lys  
       290295300

Leu Gly Gly  
305

<210> 100

<211> 306

<212> PRT

<213> Staphylococcus epidermis

<400> 100

Met Thr Arg Gln Gly Tyr Gly Glu Ser Thr Gly Lys Ile Ile Leu Met  
1                5                    10                    15

Gly Glu His Ala Val Thr Phe Gly Gln Pro Ala Ile Ala Ile Pro Phe  
          20                    25                    30

Asn Ala Gly Lys Ile Lys Val Leu Ile Glu Ser Leu Asp Glu Gly Asn  
       35                    40                    45

Tyr Ser Ser Ile Thr Ser Asp Val Tyr Asp Gly Met Leu Tyr Asp Ala  
       50                    55                    60

Pro Glu His Leu Lys Ser Ile Ile Asn Arg Phe Val Glu Lys Ser Gly  
65                    70                    75                    80

Val Lys Glu Pro Leu Ser Val Lys Ile Gln Thr Asn Leu Pro Pro Ser  
          85                    90                    95

Arg Gly Leu Gly Ser Ser Ala Ala Val Ala Val Ala Phe Val Arg Ala  
       100                    105                    110

Ser Tyr Asp Phe Met Asp Gln Pro Leu Asp Asp Lys Thr Leu Ile Lys  
       115                    120                    125

Glu Ala Asn Trp Ala Glu Gln Ile Ala His Gly Lys Pro Ser Gly Ile  
       130                    135                    140

Asp Thr Gln Thr Ile Val Ser Asn Lys Pro Val Trp Phe Lys Gln Gly  
145                    150                    155                    160

Gln Ala Glu Thr Leu Lys Ser Leu Lys Leu Asn Gly Tyr Met Val Val  
          165                    170                    175

Ile Asp Thr Gly Val Lys Gly Ser Thr Lys Gln Ala Val Glu Asp Val

[illegible]

100                      105                      110  
 Ser Tyr Asp Phe Leu Gly Lys Ser Leu Thr Lys Glu Leu Ile Glu  
     115                      120                      125  
 Lys Ala Asn Trp Ala Glu Gln Ile Ala His Gly Lys Pro Ser Gly Ile  
     130                      135                      140  
 Asp Thr Gln Thr Ile Val Ser Gly Lys Pro Val Trp Phe Gln Lys Gly  
     145                      150                      155                      160  
 Gln Ala Glu Thr Leu Lys Thr Leu Ser Leu Asp Gly Tyr Met Val Val  
     165                      170                      175  
 Ile Asp Thr Gly Val Lys Gly Ser Thr Arg Gln Ala Val Glu Asp Val  
     180                      185                      190  
 His Lys Leu Cys Glu Asp Pro Gln Tyr Met Ser His Val Lys His Ile  
     195                      200                      205  
 Gly Lys Leu Val Leu Arg Ala Ser Asp Val Ile Glu His His Asn Phe  
     210                      215                      220  
 Glu Ala Leu Ala Asp Ile Phe Asn Glu Cys His Ala Asp Leu Lys Ala  
     225                      230                      235                      240  
 Leu Thr Val Ser His Asp Lys Ile Glu Gln Leu Met Lys Ile Gly Lys  
     245                      250                      255  
 Glu Asn Gly Ala Ile Ala Gly Lys Leu Thr Gly Ala Gly Arg Gly Gly  
     260                      265                      270  
 Ser Met Leu Leu Leu Ala Lys Asp Leu Pro Thr Ala Lys Asn Ile Val  
     275                      280                      285  
 Lys Ala Val Glu Lys Ala Gly Ala Ala His Thr Trp Ile Glu Asn Leu  
     290                      295                      300  
 Gly Gly  
 305  
 <210> 102  
 <211> 345  
 <212> PRT  
 <213> Streptomyces sp. CL190  
  
 <400> 102  
 Met Gln Lys Arg Gln Arg Glu Leu Ser Ala Leu Thr Leu Pro Thr Ser  
     1                      5                      10                      15  
 Ala Glu Gly Val Ser Glu Ser His Arg Ala Arg Ser Val Gly Ile Gly

	20		25		30	
Arg	Ala	His	Ala	Lys	Ala	Ile
	35				40	
Leu	Leu	Gly	Glu	His	Ala	Val
				45		
Val	Val	Tyr				
Gly	Ala	Pro	Ala	Leu	Ala	Leu
	50			55		
Pro	Ile	Pro	Gln	Leu	Thr	Val
				60		
Thr	Val	Thr	Ala			
Ser	Val	Gly	Trp	Ser	Glu	Ala
	65			70		
Ser	Asp	Ser	Ala	Gly	Gly	Leu
				75		80
Tyr	Thr	Met	Thr	Gly	Thr	Pro
				85		
Ser	Arg	Ala	Leu	Val	Thr	Gln
				90		95
Ala	Ser					
Asp	Gly	Leu	His	Arg	Leu	Thr
		100				
Ala	Glu	Phe	Met	Ala	Arg	Met
		105				110
Thr	Asn	Ala	Pro	His	Leu	Asp
		115				
Val	Ile	Leu	Asp	Gly	Ala	Ile
		120				125
Pro	His					
Gly	Arg	Gly	Leu	Gly	Ser	Ser
		130				135
Ala	Ala	Gly	Ser	Arg	Ala	Ile
				140		
Ala	Leu	Ala	Asp	Leu	Phe	Gly
				145		
His	Glu	Leu	Ala	Glu	His	Thr
				150		
Ala	Tyr					160
Glu	Leu	Val	Gln	Thr	Ala	Glu
				165		
Asn	Met	Ala	His	Gly	Arg	Ala
				170		175
Val	Asp	Ala	Met	Thr	Val	Gly
				180		
Ala	Ser	Arg	Pro	Leu	Leu	Phe
				185		190
Gln	Gln					
Gly	Arg	Thr	Glu	Arg	Leu	Ala
				195		
Ile	Gly	Cys	Asp	Ser	Leu	Phe
				200		205
Val						
Ala	Asp	Ser	Gly	Val	Pro	Gly
				210		215
Ser	Thr	Lys	Glu	Ala	Val	Glu
				220		
Met	Leu					
Arg	Glu	Gly	Phe	Thr	Arg	Ser
				225		
Ala	Gly	Thr	Gln	Glu	Arg	Phe
				230		
Val	Gly					240
Arg	Ala	Thr	Glu	Leu	Thr	Glu
				245		
Ala	Arg	Gln	Ala	Leu	Ala	Asp
				250		255
Gly						
Arg	Pro	Glu	Glu	Leu	Gly	Ser
				260		
Gln	Leu	Thr	Tyr	Tyr	His	Glu
				265		270
Leu						
His	Glu	Ala	Arg	Leu	Ser	Thr
				275		
Asp	Gly	Ile	Asp	Ala	Leu	Val
				280		285
Ala	Leu	Lys	Ala	Gly	Ser	Leu
				290		295
Gly	Ala	Lys	Ile	Thr	Gly	Gly
				300		
Gly	Leu					
Gly	Gly	Cys	Met	Ile	Ala	Gln
				305		
Ala	Arg	Pro	Glu	Gln	Ala	Arg
				310		
Glu	Val					320
Thr	Arg	Gln	Leu	His	Glu	Ala
Gly	Ala	Val	Gln	Thr	Trp	Val
Val	Pro					

[illegible]

210                      215                      220  
 Glu Glu Arg Phe Met His Arg Ala Ala His Leu Val Asp Asp Ala Arg  
 225                      230                      235                      240  
 Ala Ser Leu Ala Glu Gly Glu Pro Glu Ala Phe Gly Ser Cys Leu Thr  
                     245                      250                      255  
 Glu Tyr His Gly Leu Leu Arg Gly Ala Gly Leu Ser Thr Asp Arg Ile  
                     260                      265                      270  
 Asp Ala Leu Val Asp Ala Ala Leu Gln Ala Asp Ser Leu Gly Ala Lys  
                     275                      280                      285  
 Ile Thr Gly Gly Gly Leu Gly Gly Cys Val Leu Ala Met Ser Arg Pro  
                     290                      295                      300  
 Glu Arg Ala Glu Glu Val Ala Arg Gln Leu His Ala Ala Gly Ala Val  
 305                      310                      315                      320  
 Arg Thr Trp Ala Val Gln Leu Arg Arg Ser Thr His Glu Arg  
                     325                      330

<210> 104

<211> 296

<212> PRT

<213> Borrelia burgdorferi

<400> 104

Met Leu Arg Ile Arg Lys Pro Ala Lys Ile Leu Phe Leu Gly Glu His  
 1                      5                      10                      15  
 Ser Ala Val Tyr Gly Phe Pro Val Ile Gly Ala Thr Val Pro Ile Tyr  
                     20                      25                      30  
 Met Asp Leu Ile Tyr Ser Val Ser Lys Asn Trp Lys Tyr Leu Gly Lys  
                     35                      40                      45  
 Pro Ser Thr Arg Leu Asn Ser Leu Ile Ser Phe Ile Val Ser Asn Tyr  
                     50                      55                      60  
 Ser Lys Val Asn Pro Ile Glu Phe Asp Ile Ile Ser Glu Ile Pro Ile  
 65                      70                      75                      80  
 Gly Val Gly Leu Gly Ser Ser Ala Ser Leu Ser Leu Cys Phe Ala Glu  
                     85                      90                      95  
 Tyr Ile Thr Ser His Phe Glu Tyr Lys Asp Cys Asn Lys Ile Leu Leu  
                     100                      105                      110  
 Ala Asn Gln Ile Glu Asn Ile Phe His Gly Lys Ser Ser Gly Met Asp

115                      120                      125  
 Ile Arg Leu Ile Asp Leu Asn Gly Thr Phe Tyr Leu Glu Lys Lys Glu  
 130                      135                      140  
 Asn Val Leu His Ser Lys Lys Ile Lys Asp Ser Gly Phe Tyr Phe Leu  
 145                      150                      155                      160  
 Ile Gly Ala Ile Lys Arg Asp Leu Thr Thr Lys Glu Ile Val Val Asn  
 165                      170                      175  
 Leu Lys Lys Asp Leu Leu Ser Asn Ala Tyr Leu Phe Val Phe Ile Glu  
 180                      185                      190  
 Lys Leu Gly Leu Ala Val Ser Asn Ser Tyr Ala Ser Phe Gln Asn Lys  
 195                      200                      205  
 Asp Val Tyr Ser Leu Ala Asn Glu Met Asn Ile Ala Gln Cys Cys Leu  
 210                      215                      220  
 Lys Arg Leu Gly Leu Ser Asn Asp Thr Leu Asp Trp Leu Ile Ser Glu  
 225                      230                      235                      240  
 Gly Ile Lys Leu Gly Ala Leu Ser Gly Lys Leu Ser Gly Ala Gly Lys  
 245                      250                      255  
 Gly Gly Ala Phe Ile Phe Leu Phe Glu Ser Leu Ile Lys Ala Asn Ile  
 260                      265                      270  
 Val Gln Lys Glu Leu Asn Asn Met Leu Asp Ser Lys Ile Asp Leu Leu  
 275                      280                      285  
 Leu Lys Leu Lys Val Ile Glu Thr  
 290                      295

&lt;210&gt; 105

&lt;211&gt; 336

&lt;212&gt; PRT

&lt;213&gt; Streptococcus pneumoniae

&lt;400&gt; 105

Met Ile Ala Val Lys Thr Cys Gly Lys Leu Tyr Trp Ala Gly Glu Tyr  
 1                      5                      10                      15  
 Ala Ile Leu Glu Pro Gly Gln Leu Ala Leu Ile Lys Asp Ile Pro Ile  
 20                      25                      30  
 Tyr Met Arg Ala Glu Ile Ala Phe Ser Asp Ser Tyr Arg Ile Tyr Ser  
 35                      40                      45  
 Asp Met Phe Asp Phe Ala Val Asp Leu Arg Pro Asn Pro Asp Tyr Ser



50	55	60
Leu Ile Gln Glu Thr	Ile Ala Leu Met Gly Asp	Phe Leu Ala Val Arg
65	70	80
Gly Gln Asn Leu Arg	Pro Phe Ser Leu Lys Ile Cys Gly Lys Met Glu	
	85	90
Arg Glu Gly Lys Lys	Phe Gly Leu Gly Ser Ser Gly Ser Val Val Val	
	100	110
Leu Val Val Lys Ala	Leu Leu Ala Leu Tyr Asn Leu Ser Val Asp Gln	
	115	120
Asn Leu Leu Phe Lys	Leu Thr Ser Ala Val Leu Leu Lys Arg Gly Asp	
	130	140
Asn Gly Ser Met Gly	Asp Leu Ala Cys Ile Val Ala Glu Asp Leu Val	
	145	155
Leu Tyr Gln Ser Phe	Asp Arg Gln Lys Ala Ala Ala Trp Leu Glu Glu	
	160	170
Glu Asn Leu Ala Thr	Val Leu Glu Arg Asp Trp Gly Phe Phe Ile Ser	
	180	190
Gln Val Lys Pro Thr	Leu Glu Cys Asp Phe Leu Val Gly Trp Thr Lys	
	195	205
Glu Val Ala Val Ser	Ser His Met Val Gln Gln Ile Lys Gln Asn Ile	
	210	220
Asn Gln Asn Phe Leu	Ser Ser Ser Lys Glu Thr Val Val Ser Leu Val	
	225	235
Glu Ala Leu Glu Gln	Gly Lys Ala Glu Lys Val Ile Glu Gln Val Glu	
	240	250
Val Ala Ser Lys Leu	Leu Glu Gly Leu Ser Thr Asp Ile Tyr Thr Pro	
	260	270
Leu Leu Arg Gln Leu	Lys Glu Ala Ser Gln Asp Leu Gln Ala Val Ala	
	275	285
Lys Ser Ser Gly Ala	Gly Gly Gly Asp Cys Gly Ile Ala Leu Ser Phe	
	290	300
Asp Ala Gln Ser Ser	Arg Asn Thr Leu Lys Asn Arg Trp Ala Asp Leu	
	305	315
Gly Ile Glu Leu Leu	Tyr Gln Glu Arg Ile Gly His Asp Asp Lys Ser	
	320	330
<210>	106	
<211>	335	

&lt;212&gt; PRT

&lt;213&gt; Streptococcus pyogenes

&lt;400&gt; 106

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Met Ser Asn Tyr Cys Val Gln Thr Gly Gly Lys Leu Tyr Leu Thr Gly
1          5          10          15
Glu Tyr Ala Ile Leu Ile Pro Gly Gln Lys Ala Leu Ile His Phe Ile
          20          25          30
Pro Leu Met Met Thr Ala Glu Ile Ser Pro Ala Ala His Ile Gln Leu
          35          40          45
Ala Ser Asp Met Phe Ser His Lys Ala Gly Met Thr Pro Asp Ala Ser
          50          55          60
Tyr Ala Leu Ile Gln Ala Thr Val Lys Thr Phe Ala Asp Tyr Leu Gly
65          70          75          80
Gln Ser Ile Asp Gln Leu Glu Pro Phe Ser Leu Ile Ile Thr Gly Lys
          85          90          95
Met Glu Arg Asp Gly Lys Lys Phe Gly Ile Gly Ser Ser Gly Ser Val
          100          105          110
Thr Leu Leu Thr Leu Lys Ala Leu Ser Ala Tyr Tyr Gln Ile Thr Leu
          115          120          125
Thr Pro Glu Leu Leu Phe Lys Leu Ala Ala Tyr Thr Leu Leu Lys Gln
          130          135          140
Gly Asp Asn Gly Ser Met Gly Asp Ile Ala Cys Ile Ala Tyr Gln Thr
145          150          155          160
Leu Val Ala Tyr Thr Ser Phe Asp Arg Glu Gln Val Ser Asn Trp Leu
          165          170          175
Gln Thr Met Pro Leu Lys Lys Leu Leu Val Lys Asp Trp Gly Tyr His
          180          185          190
Ile Gln Val Ile Gln Pro Ala Leu Pro Cys Asp Phe Leu Val Gly Trp
          195          200          205
Thr Lys Ile Pro Ala Ile Ser Arg Gln Met Ile Gln Gln Val Thr Ala
          210          215          220
Ser Ile Thr Pro Ala Phe Leu Arg Thr Ser Tyr Gln Leu Thr Gln Ser
225          230          235          240
Ala Met Val Ala Leu Gln Glu Gly His Lys Glu Glu Leu Lys Lys Ser
          245          250          255
Leu Ala Gly Ala Ser His Leu Leu Lys Glu Leu His Pro Ala Ile Tyr

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[illegible]

[illegible]

	35						40						45					
Ser	Ala	Gln	Tyr	Ser	Gly	Met	Pro	Val	Arg	Trp	Thr	Arg	Arg	Asn	Gly			
50						55					60							
Glu	Leu	Val	Leu	Asp	Ile	Arg	Glu	Asn	Pro	Phe	His	Tyr	Ile	Leu	Ala			
65					70					75					80			
Ala	Ile	Arg	Leu	Thr	Glu	Lys	Tyr	Ala	Gln	Glu	Lys	Asn	Ile	Leu	Leu			
				85					90					95				
Ser	Phe	Tyr	Asp	Leu	Lys	Val	Thr	Ser	Glu	Leu	Asp	Ser	Ser	Asn	Gly			
			100					105					110					
Arg	Lys	Tyr	Gly	Leu	Gly	Ser	Ser	Gly	Ala	Val	Thr	Val	Ala	Thr	Val			
			115				120					125						
Lys	Ala	Leu	Asn	Val	Phe	Tyr	Ala	Leu	Asn	Leu	Ser	Gln	Leu	Glu	Ile			
			130			135					140							
Phe	Lys	Ile	Ala	Ala	Leu	Ala	Asn	Leu	Ala	Val	Gln	Asp	Asn	Gly	Ser			
145				150						155					160			
Cys	Gly	Asp	Ile	Ala	Ala	Ser	Cys	Tyr	Gly	Gly	Trp	Ile	Ala	Phe	Ser			
			165						170					175				
Thr	Phe	Asp	His	Pro	Trp	Leu	Gln	Glu	Gln	Glu	Thr	Gln	His	Ser	Ile			
			180					185					190					
Ser	Glu	Leu	Leu	Ala	Leu	Asp	Trp	Pro	Gly	Leu	Ser	Ile	Glu	Pro	Leu			
			195				200					205						
Ile	Ala	Pro	Glu	Asp	Leu	Arg	Leu	Leu	Ile	Gly	Trp	Thr	Gly	Ser	Pro			
			210			215					220							
Ala	Ser	Thr	Ser	Asp	Leu	Val	Asp	Gln	Val	His	Arg	Ser	Arg	Glu	Asp			
225				230						235					240			
Lys	Met	Val	Ala	Tyr	Gln	Leu	Phe	Leu	Lys	Asn	Ser	Thr	Glu	Cys	Val			
			245						250					255				
Asn	Glu	Met	Ile	Lys	Gly	Phe	Lys	Glu	Asn	Asn	Val	Thr	Leu	Ile	Gln			
			260					265					270					
Gln	Met	Ile	Arg	Lys	Asn	Arg	Gln	Leu	Leu	His	Asp	Leu	Ser	Ala	Ile			
			275				280					285						
Thr	Gly	Val	Val	Ile	Glu	Thr	Pro	Ala	Leu	Asn	Lys	Leu	Cys	Asn	Leu			
			290			295					300							
Ala	Glu	Gln	Tyr	Glu	Gly	Ala	Ala	Lys	Ser	Ser	Gly	Ala	Gly	Gly	Gly			
305				310						315					320			
Asp	Cys	Gly	Ile	Val	Ile	Val	Asp	Gln	Lys	Ser	Gly	Ile	Leu	Pro	Leu			
			325					330						335				
Met	Ser	Ala	Trp	Glu	Lys	Ala	Glu	Ile	Thr	Pro	Leu	Pro	Leu	His	Val			

340 345 350

Tyr Ser Asp Gln Arg Lys Glu Asn Arg  
355 360

<210> 109  
<211> 358  
<212> PRT  
<213> Staphylococcus haemolyticus

<400> 109

Met Ile Gln Val Lys Ala Pro Gly Lys Leu Tyr Val Ala Gly Glu Tyr  
1 5 10 15  
Ala Val Thr Glu Pro Gly Tyr Lys Ser Val Leu Ile Ala Val Asp Arg  
20 25 30  
Phe Val Thr Ala Ser Ile Glu Ala Ser Asn Ala Val Thr Ser Thr Ile  
35 40 45  
His Ser Lys Thr Leu His Tyr Glu Pro Val Thr Phe Asn Arg Asn Glu  
50 55 60  
Asp Lys Ile Asp Ile Ser Asp Ala Asn Ala Ala Ser Gln Leu Lys Tyr  
65 70 75 80  
Val Val Thr Ala Ile Glu Val Phe Glu Gln Tyr Ala Arg Ser Cys Asn  
85 90 95  
Val Lys Leu Lys His Phe His Leu Glu Ile Asp Ser Asn Leu Asp Asp  
100 105 110  
Ala Ser Gly Asn Lys Tyr Gly Leu Gly Ser Ser Ala Ala Val Leu Val  
115 120 125  
Ser Val Val Lys Ala Leu Asn Glu Phe Tyr Asp Met Gln Leu Ser Asn  
130 135 140  
Leu Tyr Ile Tyr Lys Leu Ala Val Ile Ser Asn Met Arg Leu Gln Ser  
145 150 155 160  
Leu Ser Ser Cys Gly Asp Ile Ala Val Ser Val Tyr Ser Gly Trp Leu  
165 170 175  
Ala Tyr Ser Thr Phe Asp His Asp Trp Val Lys Gln Gln Met Glu Glu  
180 185 190  
Thr Ser Val Asn Glu Val Leu Glu Lys Asn Trp Pro Gly Leu His Ile  
195 200 205  
Glu Pro Leu Gln Ala Pro Glu Asn Met Glu Val Leu Ile Gly Trp Thr

210                      215                      220  
 Gly Ser Pro Ala Ser Ser Pro His Leu Val Ser Glu Val Lys Arg Leu  
 225                      230                      235                      240  
 Lys Ser Asp Pro Ser Phe Tyr Gly Arg Phe Leu Asp Gln Ser His Thr  
                     245                      250                      255  
 Cys Val Glu Asn Leu Ile Tyr Ala Phe Lys Thr Asp Asn Ile Lys Gly  
                     260                      265                      270  
 Val Gln Lys Met Ile Arg Gln Asn Arg Met Ile Ile Gln Gln Met Asp  
                     275                      280                      285  
 Asn Glu Ala Thr Val Asp Ile Glu Thr Glu Asn Leu Lys Met Leu Cys  
                     290                      295                      300  
 Asp Ile Gly Glu Arg Tyr Gly Ala Ala Ala Lys Thr Ser Gly Ala Gly  
 305                      310                      315                      320  
 Gly Gly Asp Cys Gly Ile Ala Ile Ile Asp Asn Arg Ile Asp Lys Asn  
                     325                      330                      335  
 Arg Ile Tyr Asn Glu Trp Ala Ser His Gly Ile Lys Pro Leu Lys Phe  
                     340                      345                      350  
 Lys Ile Tyr His Gly Gln  
                     355  
 <210> 110  
 <211> 358  
 <212> PRT  
 <213> Staphylococcus epidermis  
  
 <400> 110  
 Met Ile Gln Val Lys Ala Pro Gly Lys Leu Tyr Ile Ala Gly Glu Tyr  
 1                      5                      10                      15  
 Ala Val Thr Glu Pro Gly Tyr Lys Ser Ile Leu Ile Ala Val Asn Arg  
                     20                      25                      30  
 Phe Val Thr Ala Thr Ile Glu Ala Ser Asn Lys Val Glu Gly Ser Ile  
                     35                      40                      45  
 His Ser Lys Thr Leu His Tyr Glu Pro Val Lys Phe Asp Arg Asn Glu  
                     50                      55                      60  
 Asp Arg Ile Glu Ile Ser Asp Val Gln Ala Ala Lys Gln Leu Lys Tyr  
 65                      70                      75                      80  
 Val Val Thr Ala Ile Glu Val Phe Glu Gln Tyr Val Arg Ser Cys Asn

85										90					95				
Met	Asn	Leu	Lys	His	Phe	His	Leu	Thr	Ile	Asp	Ser	Asn	Leu	Ala	Asp				
100										105					110				
Asn	Ser	Gly	Gln	Lys	Tyr	Gly	Leu	Gly	Ser	Ser	Ala	Ala	Val	Leu	Val				
115										120					125				
Ser	Val	Val	Lys	Ala	Leu	Asn	Glu	Phe	Tyr	Gly	Leu	Glu	Leu	Ser	Asn				
130										135					140				
Leu	Tyr	Ile	Tyr	Lys	Leu	Ala	Val	Ile	Ala	Asn	Met	Lys	Leu	Gln	Ser				
145										150					155				
Leu	Ser	Ser	Cys	Gly	Asp	Ile	Ala	Val	Ser	Val	Tyr	Ser	Gly	Trp	Leu				
165										170					175				
Ala	Tyr	Ser	Thr	Phe	Asp	His	Asp	Trp	Val	Lys	Gln	Gln	Met	Glu	Glu				
180										185					190				
Thr	Ser	Val	Asn	Asp	Val	Leu	Glu	Lys	Asn	Trp	Pro	Gly	Leu	His	Ile				
195										200					205				
Glu	Pro	Leu	Gln	Ala	Pro	Glu	Asn	Met	Glu	Val	Leu	Ile	Gly	Trp	Thr				
210										215					220				
Gly	Ser	Pro	Ala	Ser	Ser	Pro	His	Leu	Val	Ser	Glu	Val	Lys	Arg	Leu				
225										230					235				
Lys	Ser	Asp	Pro	Ser	Phe	Tyr	Gly	Asp	Phe	Leu	Asp	Gln	Ser	His	Ala				
245										250					255				
Cys	Val	Glu	Ser	Leu	Ile	Gln	Ala	Phe	Lys	Thr	Asn	Asn	Ile	Lys	Gly				
260										265					270				
Val	Gln	Lys	Met	Ile	Arg	Ile	Asn	Arg	Arg	Ile	Ile	Gln	Ser	Met	Asp				
275										280					285				
Asn	Glu	Ala	Ser	Val	Glu	Ile	Glu	Thr	Asp	Lys	Leu	Lys	Lys	Leu	Cys				
290										295					300				
Asp	Val	Gly	Glu	Lys	His	Gly	Gly	Ala	Ser	Lys	Thr	Ser	Gly	Ala	Gly				
305										310					315				
Gly	Gly	Asp	Cys	Gly	Ile	Thr	Ile	Ile	Asn	Lys	Val	Ile	Asp	Lys	Asn				
325										330					335				
Ile	Ile	Tyr	Asn	Glu	Trp	Gln	Met	Asn	Asp	Ile	Lys	Pro	Leu	Lys	Phe				
340										345					350				
Lys	Ile	Tyr	His	Gly	Gln														
355																			
<210> 111																			
<211> 358																			



&lt;212&gt; PRT

<213> *Staphylococcus aureus*

&lt;400&gt; 111

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Met Ile Gln Val Lys Ala Pro Gly Lys Leu Tyr Ile Ala Gly Glu Tyr
 1              5              10              15
Ala Val Thr Glu Pro Gly Tyr Lys Ser Val Leu Ile Ala Leu Asp Arg
 20              25              30
Phe Val Thr Ala Thr Ile Glu Glu Ala Thr Gln Tyr Lys Gly Thr Ile
 35              40              45
His Ser Lys Ala Leu His His Asn Pro Val Thr Phe Ser Arg Asp Glu
 50              55              60
Asp Ser Ile Val Ile Ser Asp Pro His Ala Ala Lys Gln Leu Asn Tyr
 65              70              75              80
Val Val Thr Ala Ile Glu Ile Phe Glu Gln Tyr Ala Lys Ser Cys Asp
 85              90              95
Ile Ala Met Lys His Phe His Leu Thr Ile Asp Ser Asn Leu Asp Asp
100              105              110
Ser Asn Gly His Lys Tyr Gly Leu Gly Ser Ser Ala Ala Val Leu Val
115              120              125
Ser Val Ile Lys Val Leu Asn Glu Phe Tyr Asp Met Lys Leu Ser Asn
130              135              140
Leu Tyr Ile Tyr Lys Leu Ala Val Ile Ala Asn Met Lys Leu Gln Ser
145              150              155              160
Leu Ser Ser Cys Gly Asp Ile Ala Val Ser Val Tyr Ser Gly Trp Leu
165              170              175
Ala Tyr Ser Thr Phe Asp His Glu Trp Val Lys His Gln Ile Glu Asp
180              185              190
Thr Thr Val Glu Glu Val Leu Ile Lys Asn Trp Pro Gly Leu His Ile
195              200              205
Glu Pro Leu Gln Ala Pro Glu Asn Met Glu Val Leu Ile Gly Trp Thr
210              215              220
Gly Ser Pro Ala Ser Ser Pro His Phe Val Ser Glu Val Lys Arg Leu
225              230              235              240
Lys Ser Asp Pro Ser Phe Tyr Gly Asp Phe Leu Glu Asp Ser His Arg
245              250              255
Cys Val Glu Lys Leu Ile His Ala Phe Lys Thr Asn Asn Ile Lys Gly

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260                      265                      270  
 Val Gln Lys Met Val Arg Gln Asn Arg Thr Ile Ile Gln Arg Met Asp  
                          275                      280                      285  
 Lys Glu Ala Thr Val Asp Ile Glu Thr Glu Lys Leu Lys Tyr Leu Cys  
                          290                      295                      300  
 Asp Ile Ala Glu Lys Tyr His Gly Ala Ser Lys Thr Ser Gly Ala Gly  
                          305                      310                      315                      320  
 Gly Gly Asp Cys Gly Ile Thr Ile Ile Asn Lys Asp Val Asp Lys Glu  
                          325                      330                      335  
 Lys Ile Tyr Asp Glu Trp Thr Lys His Gly Ile Lys Pro Leu Lys Phe  
                          340                      345                      350  
 Asn Ile Tyr His Gly Gln  
                          355

&lt;210&gt; 112

&lt;211&gt; 374

&lt;212&gt; PRT

&lt;213&gt; Streptomyces sp. CL190

&lt;400&gt; 112

Met Thr Thr Gly Gln Arg Thr Ile Val Arg His Ala Pro Gly Lys Leu  
 1                      5                      10                      15  
 Phe Val Ala Gly Glu Tyr Ala Val Val Asp Pro Gly Asn Pro Ala Ile  
                          20                      25                      30  
 Leu Val Ala Val Asp Arg His Ile Ser Val Thr Val Ser Asp Ala Asp  
                          35                      40                      45  
 Ala Asp Thr Gly Ala Ala Asp Val Val Ile Ser Ser Asp Leu Gly Pro  
                          50                      55                      60  
 Gln Ala Val Gly Trp Arg Trp His Asp Gly Arg Leu Val Val Arg Asp  
 65                      70                      75                      80  
 Pro Asp Asp Gly Gln Gln Ala Arg Ser Ala Leu Ala His Val Val Ser  
                          85                      90                      95  
 Ala Ile Glu Thr Val Gly Arg Leu Leu Gly Glu Arg Gly Gln Lys Val  
                          100                      105                      110  
 Pro Ala Leu Thr Leu Ser Val Ser Ser Arg Leu His Glu Asp Gly Arg  
                          115                      120                      125  
 Lys Phe Gly Leu Gly Ser Ser Gly Ala Val Thr Val Ala Thr Val Ala

130                      135                      140  
 Ala Val Ala Ala Phe Cys Gly Leu Glu Leu Ser Thr Asp Glu Arg Phe  
 145                      150                      155                      160  
 Arg Leu Ala Met Leu Ala Thr Ala Glu Leu Asp Pro Lys Gly Ser Gly  
                     165                      170                      175  
 Gly Asp Leu Ala Ala Ser Thr Trp Gly Gly Trp Ile Ala Tyr Gln Ala  
                     180                      185                      190  
 Pro Asp Arg Ala Phe Val Leu Asp Leu Ala Arg Arg Val Gly Val Asp  
                     195                      200                      205  
 Arg Thr Leu Lys Ala Pro Trp Pro Gly His Ser Val Arg Arg Leu Pro  
                     210                      215                      220  
 Ala Pro Lys Gly Leu Thr Leu Glu Val Gly Trp Thr Gly Glu Pro Ala  
 225                      230                      235                      240  
 Ser Thr Ala Ser Leu Val Ser Asp Leu His Arg Arg Thr Trp Arg Gly  
                     245                      250                      255  
 Ser Ala Ser His Gln Arg Phe Val Glu Thr Thr Thr Asp Cys Val Arg  
                     260                      265                      270  
 Ser Ala Val Thr Ala Leu Glu Ser Gly Asp Asp Thr Ser Leu Leu His  
                     275                      280                      285  
 Glu Ile Arg Arg Ala Arg Gln Glu Leu Ala Arg Leu Asp Asp Glu Val  
                     290                      295                      300  
 Gly Leu Gly Ile Phe Thr Pro Lys Leu Thr Ala Leu Cys Asp Ala Ala  
 305                      310                      315                      320  
 Glu Ala Val Gly Gly Ala Ala Lys Pro Ser Gly Ala Gly Gly Gly Asp  
                     325                      330                      335  
 Cys Gly Ile Ala Leu Leu Asp Ala Glu Ala Ser Arg Asp Ile Thr His  
                     340                      345                      350  
 Val Arg Gln Arg Trp Glu Thr Ala Gly Val Leu Pro Leu Pro Leu Thr  
                     355                      360                      365  
 Pro Ala Leu Glu Gly Ile  
                     370  
 <210> 113  
 <211> 360  
 <212> PRT  
 <213> *Streptomyces griseolosporeus*

&lt;400&gt; 113

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Met Thr Gly Pro Arg Ala Val Thr Arg Arg Ala Pro Gly Lys Leu Phe
 1              5              10              15

Val Ala Gly Glu Tyr Ala Val Val Glu Pro Gly Asn Arg Ala Ile Leu
      20              25              30

Val Ala Val Asp Arg Tyr Val Thr Val Thr Val Ser Asp Gly Ala Ala
      35              40              45

Pro Gly Val Val Val Ser Ser Asp Ile Gly Ala Gly Pro Val His His
      50              55              60

Pro Trp Gln Asp Gly Arg Leu Thr Gly Gly Thr Gly Thr Pro His Val
      65              70              75              80

Val Ala Ala Val Glu Thr Val Ala Arg Leu Leu Ala Glu Arg Gly Arg
      85              90              95

Ser Val Pro Pro Leu Gly Trp Ser Ile Ser Ser Thr Leu His Glu Asp
      100              105              110

Gly Arg Lys Phe Gly Leu Gly Ser Ser Gly Ala Val Thr Val Ala Thr
      115              120              125

Val Ser Ala Val Ala Ala His Cys Gly Leu Glu Leu Thr Ala Glu Glu
      130              135              140

Arg Phe Arg Thr Ala Leu Ile Ala Ser Ala Arg Ile Asp Pro Arg Gly
      145              150              155              160

Ser Gly Gly Asp Ile Ala Thr Ser Thr Trp Gly Gly Trp Ile Ala Tyr
      165              170              175

Arg Ala Pro Asp Arg Asp Ala Val Leu Asp Leu Thr Arg Arg Gln Gly
      180              185              190

Val Asp Glu Ala Leu Arg Ala Pro Trp Pro Gly Phe Ser Val Arg Leu
      195              200              205

Ser Pro Pro Arg Asn Leu Cys Leu Glu Val Gly Trp Thr Gly Asn Pro
      210              215              220

Val Ser Thr Thr Ser Leu Leu Thr Asp Leu His Arg Arg Thr Trp Arg
      225              230              235              240

Gly Ser Pro Ala Tyr Arg Arg Tyr Val Gly Ala Thr Gly Glu Leu Val
      245              250              255

Asp Ala Ala Val Ile Ala Leu Glu Asp Gly Asp Thr Glu Gly Leu Leu
      260              265              270

Arg Gln Val Arg Arg Ala Arg His Glu Met Val Arg Leu Asp Asp Glu
      275              280              285

Val Gly Leu Gly Ile Phe Thr Pro Glu Leu Thr Ala Leu Cys Ala Ile

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290                      295                      300  
 Ala Glu Arg Ala Gly Ala Ala Lys Pro Ser Gly Ala Gly Gly Gly Asp  
 305                      310                      315                      320  
 Cys Gly Ile Ala Leu Leu Asp Ala Glu Ala Arg Tyr Asp Arg Ser Pro  
                     325                      330                      335  
 Leu His Arg Gln Trp Ala Ala Ala Gly Val Leu Pro Leu Leu Val Ser  
                     340                      345                      350  
 Pro Ala Thr Glu Gly Val Glu Glu  
                     355                      360  
 <210> 114  
 <211> 317  
 <212> PRT  
 <213> *Borrelia burgdorferi*  
  
 <400> 114  
 Met Asp Leu Ile Ser Phe Ser Val Pro Gly Asn Leu Leu Leu Met Gly  
 1                      5                      10                      15  
 Glu Tyr Thr Ile Leu Glu Glu Lys Gly Leu Gly Leu Ala Ile Ala Ile  
                     20                      25                      30  
 Asn Lys Arg Ala Phe Phe Ser Phe Lys Lys Ser Asp Ser Trp Arg Phe  
                     35                      40                      45  
 Phe Ser Lys Lys Lys Lys Ile Asp Asp Phe Ser Leu Ile Glu Asn Arg  
                     50                      55                      60  
 Ser Asp Phe Val Phe Lys Met Phe Ala Tyr Leu Ser Gln Asn Cys Phe  
 65                      70                      75                      80  
 Phe Asn Leu Glu Asn Phe Ala Tyr Asp Val Tyr Ile Asp Thr Ser Asn  
                     85                      90                      95  
 Phe Phe Phe Asn Asp Gly Thr Lys Lys Gly Phe Gly Ser Ser Ala Val  
                     100                      105                      110  
 Val Ala Ile Gly Ile Val Cys Gly Leu Phe Leu Ile His Asn Ala Thr  
                     115                      120                      125  
 Asn Val Val Glu Lys Gly Glu Ile Phe Lys Tyr Cys Leu Glu Ala Tyr  
                     130                      135                      140  
 Arg Tyr Ser Gln Gly Gly Ile Gly Ser Gly Tyr Asp Ile Ala Thr Ser  
 145                      150                      155                      160  
 Ile Phe Gly Gly Val Ile Glu Phe Glu Gly Gly Phe Asn Pro Lys Cys

165										170					175							
Arg	Gln	Leu	Gly	Ala	Val	Glu	Phe	Asn	Asp	Phe	Tyr	Leu	Met	Gln	Gly							
180										185					190							
Leu	Gln	Ala	Ile	Lys	Thr	Thr	Thr	Ser	Ile	Cys	Glu	Tyr	Asn	Lys	His							
195										200					205							
Arg	Asn	Ser	Ile	Leu	Asp	Phe	Ile	Leu	Lys	Cys	Asn	Leu	Glu	Met	Lys							
210										215					220							
Lys	Leu	Val	Leu	Asn	Ala	Ser	Asn	Ser	Lys	Ser	Ala	Leu	Ile	Ser	Ser							
225										230					235							
Leu	Arg	Arg	Ala	Lys	Glu	Leu	Gly	Leu	Ala	Ile	Gly	Glu	Ala	Ile	Gly							
240										245					250							
Val	Ser	Ala	Ala	Leu	Pro	Ser	Ser	Phe	Asp	His	Leu	Leu	Gly	Gln	Cys							
255										260					265							
Asp	Leu	Ile	Lys	Ala	Leu	Gly	Ala	Gly	Asn	Glu	Thr	Phe	Leu	Val	Tyr							
270										275					280							
Arg	Pro	Asn	Ile	Glu	Ala	Phe	Asn	Leu	Ser	Lys	Ile	Ile	Ser	Ile	Val							
285										290					295							
Leu	Glu	Asn	Glu	Gly	Ile	Lys	Phe	Glu	Ser	Asp	Lys	Cys										
300										305					310							
315										320					325							

<210> 115

&lt;211&gt; 30

<212> DNA

<213> synthetic construct

<400> 115  
gggcaagctt gtccacggca cgaccaagca

30

<210> 116

<211> 30

<212> DNA

<213> synthetic construct

<400> 116  
cgtaatccgc gccgcgctt ccagcgcgtc

30

<210> 117

<211> 28

<212> DNA

<213> synthetic construct

<400> 117

aattaaagga gggtttcata tgaattcg

28

<210> 118

<211> 28

<212> DNA

<213> synthetic construct

<400> 118

gatccgaatt catatgaaac cctccttt

28

<210> 119

<211> 30

<212> DNA

<213> synthetic construct

<400> 119

aaggcctcat atgatttccc ataccccggt

30

<210> 120

<211> 28

<212> DNA

<213> synthetic construct

<400> 120

cgggatcctc atcgtccat ctccatgt

28

<210> 121

<211> 30

<212> DNA

<213> synthetic construct

<400> 121

aaggcctcat atgaccgaca gcaaggatca

30

<210> 122

<211> 28

<212> DNA

<213> synthetic construct

<400> 122

cgggatcctc attgacggat aagcgagg

28

<210> 123

<211> 29

<212> DNA

<213> synthetic construct

<400> 123

aaggcctcat atgaaagtgc ctaagatga

29

<210> 124

<211> 28

<212> DNA

<213> synthetic construct

<400> 124

cgggatcctc aggcctgccg gtcgacat

28

<210> 125



<211> 34

<212> DNA

<213> synthetic construct

<400> 125

aaggcctcat atgagcaccg gcaggcctga agca

34

<210> 126

<211> 31

<212> DNA

<213> synthetic construct

<400> 126

cgggatactc atccctgcc cggcagcggt t

31

<210> 127

<211> 30

<212> DNA

<213> synthetic construct

<400> 127

aaggcctcat atggatcagg tcatccgcgc

30

<210> 128

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28

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28

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31

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31

<210> 136

<211> 31

<212> DNA

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31

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ggaattcgct gctgaacgcy atggcg 26

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<210> 157  
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&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (59) .. (292)

&lt;223&gt; XseB

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1185) .. (1610)

&lt;223&gt; IspA

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (295) .. (1158)

&lt;223&gt; Dxs

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Met Ser Asp Ile Gln Thr Leu Ser Phe Glu Glu Ala Met Arg Glu Leu
1          5          10          15

gag gcg acc gtc ggc aag ctg gaa acc ggc gag gcg acg ctc gag gac      154
Glu Ala Thr Val Gly Lys Leu Glu Thr Gly Glu Ala Thr Leu Glu Asp
20          25          30

tcc atc gcg ctc tat gaa cgc ggg gcg gcg ctg cgc gcc cat tgc gaa      202
Ser Ile Ala Leu Tyr Glu Arg Gly Ala Ala Leu Arg Ala His Cys Glu
35          40          45

acc cgc ctg cgc gag gcc gag gag cgg gtc gag aag atc acc ctg gcc      250
Thr Arg Leu Arg Glu Ala Glu Glu Arg Val Glu Lys Ile Thr Leu Ala
50          55          60

gcg aac ggg cag ccg tcc gga acc gag ccc gcc gag ggc ctg tg atg      297
Ala Asn Gly Gln Pro Ser Gly Thr Glu Pro Ala Glu Gly Leu Met
65          70          75

cag gcc cgc ctg gcc gag atc cgg ccc ctg gtc gag gcc gag ctg aac      345
Gln Ala Arg Leu Ala Glu Ile Arg Pro Leu Val Glu Ala Glu Leu Asn
80          85          90          95

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gcc gcc atc gac gcg ctg ccc gcg ggc gat ctg tcg gat gcg atg cgc Ala Ala Ile Asp Ala Leu Pro Ala Gly Asp Leu Ser Asp Ala Met Arg 100 105 110	393
tat gcc gtg cag ggc ggc aag cgg ctg cgc gcg ttc ctg gtg atg gag Tyr Ala Val Gln Gly Lys Arg Leu Arg Ala Phe Leu Val Met Glu 115 120 125	441
tcg gcg cgc ctg cac ggg ctg gac gac gac gca tcg ctg ccc gtc gcc Ser Ala Arg Leu His Gly Leu Asp Asp Asp Ala Ser Leu Pro Val Ala 130 135 140	489
gcc gcg gtc gag gcg ctg cac gcc tac agc ttg gtc cat gac gac ctg Ala Ala Val Glu Ala Leu His Ala Tyr Ser Leu Val His Asp Asp Leu 145 150 155	537
ccc gcg atg gat gac gac gac ctg cgg cgc ggt cag ccc acc gtc cac Pro Ala Met Asp Asp Asp Asp Leu Arg Arg Gly Gln Pro Thr Val His 160 165 170 175	585
gtc aaa tgg acc gag gcg acc gcg atc ctt gcg ggc gat gcg ctg cag Val Lys Trp Thr Glu Ala Thr Ala Ile Leu Ala Gly Asp Ala Leu Gln 180 185 190	633
acg ctg gcc ttc cag ctg ctg gcc gat cgc gcg gtg ggc gac gat gcg Thr Leu Ala Phe Gln Leu Leu Ala Asp Pro Arg Val Gly Asp Asp Ala 195 200 205	681
gcg cgg atg cgg ctg gtc ggt tcg ctg gcg cag gca tcg ggg gct gcg Ala Arg Met Arg Leu Val Gly Ser Leu Ala Gln Ala Ser Gly Ala Ala 210 215 220	729
ggc atg gtc tgg ggc cag gcg ctg gac atc gcg gcc gag acc tcg ggc Gly Met Val Trp Gly Gln Ala Leu Asp Ile Ala Ala Glu Thr Ser Gly 225 230 235	777
gtg cgg ctg gat ctg gac gcg atc atc cgc ctg cag ggt ggc aag acc Val Pro Leu Asp Leu Asp Ala Ile Ile Arg Leu Gln Gly Gly Lys Thr 240 245 250 255	825
ggc gcg ctg atc cgc ttt gcc gcg acc gcc ggg cgc ctg atg gcg ggg Gly Ala Leu Ile Arg Phe Ala Ala Thr Ala Gly Pro Leu Met Ala Gly 260 265 270	873
gcg gac cct gcc gcg ctg gac gat tat gcg cag gcc gtc ggg ctg gcc Ala Asp Pro Ala Ala Leu Asp Asp Tyr Ala Gln Ala Val Gly Leu Ala 275 280 285	921
ttc cag atc gcg gac gac atc ctg gac gtc gag ggc tgc gag gcc gcg Phe Gln Ile Ala Asp Asp Ile Leu Asp Val Glu Gly Cys Glu Ala Ala 290 295 300	969
acc ggc aag cgc gtc ggc aag gat gcg gat gcc aac aag gcg acc ttc Thr Gly Lys Arg Val Gly Lys Asp Ala Asp Ala Asn Lys Ala Thr Phe 305 310 315	1017
gtc tcg ctg ctg ggc ctc gag ggg gcg cgg tcc gag gcg cgt cgc ctg	1065

Val Ser Leu Leu Gly Leu Glu Gly Ala Arg Ser Glu Ala Arg Arg Leu 320 325 330 335	
gcc gat gcg ggg cag gac gcg ctg gcg ggt tac ggc gat gct gcg ggg Ala Asp Ala Gly Gln Asp Ala Leu Ala Gly Tyr Gly Asp Ala Ala Gly 340 345 350	1113
aac ctt cgg gac ctg gcg cgc ttc gtg atc gaa cgc gac agc tga Asn Leu Arg Asp Leu Ala Arg Phe Val Ile Glu Arg Asp Ser 355 360 365	1158
tcgccgcctt ccgcaccaagg ggcaag atg atg acc gac gga ccc gca acc ccg Met Met Thr Asp Gly Pro Ala Thr Pro 370	1211
atc ctg gac cgc gtc cag cag cca tcc gac ctg gca tcg ctg gac gat Ile Leu Asp Arg Val Gln Gln Pro Ser Asp Leu Ala Ser Leu Asp Asp 375 380 385 390	1259
gcg cag ctg cgc ctg ctg gcg gac gag ctg cgg gcc gag acc atc gac Ala Gln Leu Arg Leu Leu Ala Asp Glu Leu Arg Ala Glu Thr Ile Asp 395 400 405	1307
atc gtc agc cgc acg ggc ggt cac ctg ggc gcg ggg ctg ggc gtg gtc Ile Val Ser Arg Thr Gly Gly His Leu Gly Ala Gly Leu Gly Val Val 410 415 420	1355
gaa ctg acg gtc gcc ctg cac gcc gtc ttt cgg gcg ccg cgc gac aag Glu Leu Thr Val Ala Leu His Ala Val Phe Arg Ala Pro Arg Asp Lys 425 430 435	1403
atc gtc tgg gac gtg ggg cat caa tgc tat ccc cac aag atc ctg acg Ile Val Trp Asp Val Gly His Gln Cys Tyr Pro His Lys Ile Leu Thr 440 445 450	1451
ggc agg cgg gac cgg atg cgc acg ctg cgc atg ggc ggc ggg ctg tcg Gly Arg Arg Asp Arg Met Arg Thr Leu Arg Met Gly Gly Gly Leu Ser 455 460 465 470	1499
ggg ttc acc aag cgg cag gaa agc gcg ttc gat ccg ttc ggt gcg ggg Gly Phe Thr Lys Arg Gln Glu Ser Ala Phe Asp Pro Phe Gly Ala Gly 475 480 485	1547
cac agc tcg acc tcg atc tcg gcg gcg ctg ggc ttc gcg atg gcg cgt His Ser Ser Thr Ser Ile Ser Ala Ala Leu Gly Phe Ala Met Ala Arg 490 495 500	1595
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<212> PRT	

<213> Paracoccus sp. R114

<400> 158

Met Ser Asp Ile Gln Thr Leu Ser Phe Glu Glu Ala Met Arg Glu Leu  
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Glu Ala Thr Val Gly Lys Leu Glu Thr Gly Glu Ala Thr Leu Glu Asp  
20 25 30

Ser Ile Ala Leu Tyr Glu Arg Gly Ala Ala Leu Arg Ala His Cys Glu  
35 40 45

Thr Arg Leu Arg Glu Ala Glu Glu Arg Val Glu Lys Ile Thr Leu Ala  
50 55 60

Ala Asn Gly Gln Pro Ser Gly Thr Glu Pro Ala Glu Gly Leu  
65 70 75

<210> 159

<211> 287

<212> PRT

<213> Paracoccus sp. R114

<400> 159

Met Gln Ala Arg Leu Ala Glu Ile Arg Pro Leu Val Glu Ala Glu Leu  
1 5 10 15

Asn Ala Ala Ile Asp Ala Leu Pro Ala Gly Asp Leu Ser Asp Ala Met  
20 25 30

Arg Tyr Ala Val Gln Gly Gly Lys Arg Leu Arg Ala Phe Leu Val Met  
35 40 45

Glu Ser Ala Arg Leu His Gly Leu Asp Asp Ala Ser Leu Pro Val  
50 55 60

Ala Ala Ala Val Glu Ala Leu His Ala Tyr Ser Leu Val His Asp Asp  
65 70 75 80

Leu Pro Ala Met Asp Asp Asp Asp Leu Arg Arg Gly Gln Pro Thr Val  
 85 90 95  
 His Val Lys Trp Thr Glu Ala Thr Ala Ile Leu Ala Gly Asp Ala Leu  
 100 105 110  
 Gln Thr Leu Ala Phe Gln Leu Leu Ala Asp Pro Arg Val Gly Asp Asp  
 115 120 125  
 Ala Ala Arg Met Arg Leu Val Gly Ser Leu Ala Gln Ala Ser Gly Ala  
 130 135 140  
 Ala Gly Met Val Trp Gly Gln Ala Leu Asp Ile Ala Ala Glu Thr Ser  
 145 150 155 160  
 Gly Val Pro Leu Asp Leu Asp Ala Ile Ile Arg Leu Gln Gly Gly Lys  
 165 170 175  
 Thr Gly Ala Leu Ile Arg Phe Ala Ala Thr Ala Gly Pro Leu Met Ala  
 180 185 190  
 Gly Ala Asp Pro Ala Ala Leu Asp Asp Tyr Ala Gln Ala Val Gly Leu  
 195 200 205  
 Ala Phe Gln Ile Ala Asp Asp Ile Leu Asp Val Glu Gly Cys Glu Ala  
 210 215 220  
 Ala Thr Gly Lys Arg Val Gly Lys Asp Ala Asp Ala Asn Lys Ala Thr  
 225 230 235 240  
 Phe Val Ser Leu Leu Gly Leu Glu Gly Ala Arg Ser Glu Ala Arg Arg  
 245 250 255  
 Leu Ala Asp Ala Gly Gln Asp Ala Leu Ala Gly Tyr Gly Asp Ala Ala  
 260 265 270  
 Gly Asn Leu Arg Asp Leu Ala Arg Phe Val Ile Glu Arg Asp Ser  
 275 280 285

&lt;210&gt; 160

&lt;211&gt; 142

&lt;212&gt; PRT

<213> *Paracoccus* sp. R114

&lt;400&gt; 160

Met Met Thr Asp Gly Pro Ala Thr Pro Ile Leu Asp Arg Val Gln Gln  
 1 5 10 15

Pro Ser Asp Leu Ala Ser Leu Asp Asp Ala Gln Leu Arg Leu Leu Ala  
 20 25 30

Asp Glu Leu Arg Ala Glu Thr Ile Asp Ile Val Ser Arg Thr Gly Gly  
 35 40 45

His Leu Gly Ala Gly Leu Gly Val Val Glu Leu Thr Val Ala Leu His  
 50 55 60

Ala Val Phe Arg Ala Pro Arg Asp Lys Ile Val Trp Asp Val Gly His  
 65 70 75 80

Gln Cys Tyr Pro His Lys Ile Leu Thr Gly Arg Arg Asp Arg Met Arg  
 85 90 95

Thr Leu Arg Met Gly Gly Gly Leu Ser Gly Phe Thr Lys Arg Gln Glu  
 100 105 110

Ser Ala Phe Asp Pro Phe Gly Ala Gly His Ser Ser Thr Ser Ile Ser  
 115 120 125

Ala Ala Leu Gly Phe Ala Met Ala Arg Glu Leu Gly Gly Asp  
 130 135 140

&lt;210&gt; 161

&lt;211&gt; 6

&lt;212&gt; PRT

<213> *Bradyrhizobium japonicum*

&lt;400&gt; 161

Val His Asp Asp Leu Pro  
1 5

<210> 162

<211> 6

<212> PRT

<213> Rhizobium sp. strain NGR234

<400> 162

Val His Asp Asp Leu Pro  
1 5

<210> 163

<211> 6

<212> PRT

<213> Bacillus stearothermophilus

<400> 163

Ile His Asp Asp Leu Pro  
1 5

<210> 164

<211> 6

<212> PRT

<213> Bacillus subtilis

<400> 164

Ile His Asp Asp Leu Pro  
1 5

<210> 165

<211> 6

<212> PRT

<213> Escherichia coli

<400> 165

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1 5

<210> 166

<211> 6

<212> PRT

<213> Haemophilus influenzae

<400> 166

Ile His Asp Asp Leu Pro  
1 5

<210> 167

<211> 16

<212> DNA

<213> synthetic construct

<400> 167

tccaygayga yctgcc

16

<210> 168

<211> 5

<212> PRT

<213> Bradyrhizobium japonicum

<400> 168

Asp Asp Ile Leu Asp  
1 5

<210> 169

<211> 5

<212> PRT

<213> Rhizobium sp. strain NGR234



<400> 169

Asp Asp Ile Leu Asp  
1 5

<210> 170

<211> 5

<212> PRT

<213> *Bacillus stearothermophilus*

<400> 170

Asp Asp Ile Leu Asp  
1 5

<210> 171

<211> 5

<212> PRT

<213> *Bacillus subtilis*

<400> 171

Asp Asp Ile Leu Asp  
1 5

<210> 172

<211> 5

<212> PRT

<213> *Escherichia coli*

<400> 172

Asp Asp Ile Leu Asp  
1 5

<210> 173

<211> 5

&lt;212&gt; PRT

&lt;213&gt; Haemophilus influenzae

&lt;400&gt; 173

Asp Asp Ile Leu Asp  
1 5

&lt;210&gt; 174

&lt;211&gt; 15

&lt;212&gt; DNA

&lt;213&gt; synthetic construct

&lt;400&gt; 174

gaygayatcc tggay

15

&lt;210&gt; 175

&lt;211&gt; 1176

&lt;212&gt; DNA

&lt;213&gt; Paracoccus sp. R1534

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(1173)

&lt;223&gt; acety-CoA acetyltransferase

&lt;400&gt; 175

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Met Asp Pro Ile Val Ile Thr Gly Ala Met Arg Thr Pro Met Gly Ala  
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ttc cag ggc gat ctt gcc gcg atg gat gcc ccg acc ctt ggc gcg gcc 96  
Phe Gln Gly Asp Leu Ala Ala Met Asp Ala Pro Thr Leu Gly Ala Ala  
20 25 30

gcg atc cgc gcc gcg ctg aac ggc ctg tcg ccc gac atg gtg gac gag 144  
Ala Ile Arg Ala Ala Leu Asn Gly Leu Ser Pro Asp Met Val Asp Glu  
35 40 45

gtg ctg atg ggc tgc gtc ctg ccc gcg ggc cag ggt cag gca ccg gca Val Leu Met Gly Cys Val Leu Pro Ala Gly Gln Gly Gln Ala Pro Ala 50 55 60	192
cgt cag gcg gcg ctt gac gcc gga ctg ccg ctg tcg gcg ggc gcg acc Arg Gln Ala Ala Leu Asp Ala Gly Leu Pro Leu Ser Ala Gly Ala Thr 65 70 75 80	240
acc atc aac aag atg tgc gga tcg ggc atg aag gcc gcg atg ctg ggc Thr Ile Asn Lys Met Cys Gly Ser Gly Met Lys Ala Ala Met Leu Gly 85 90 95	288
cat gac ctg atc gcc gcg gga tcg gcg ggc atc gtc gtc gcc ggc ggg His Asp Leu Ile Ala Ala Gly Ser Ala Gly Ile Val Val Ala Gly Gly 100 105 110	336
atg gag agc atg tcg aac gcc ccc tac ctg ctg ccc aag gcg ccg tcg Met Glu Ser Met Ser Asn Ala Pro Tyr Leu Leu Pro Lys Ala Arg Ser 115 120 125	384
ggg atg cgc atg ggc cat gac cgt gtg ctg gat cac atg ttc ctc gac Gly Met Arg Met Gly His Asp Arg Val Leu Asp His Met Phe Leu Asp 130 135 140	432
ggg ttg gag gac gcc tat gac aag ggc cgc ctg atg ggc acc ttc gcc Gly Leu Glu Asp Ala Tyr Asp Lys Gly Arg Leu Met Gly Thr Phe Ala 145 150 155 160	480
gag gat tgc gcc ggc gat cac ggt ttc acc cgc gag gcg cag gac gac Glu Asp Cys Ala Gly Asp His Gly Phe Thr Arg Glu Ala Gln Asp Asp 165 170 175	528
tat gcg ctg acc agc ctg gcc cgc gcg cag gac gcc atc gcc agc ggt Tyr Ala Leu Thr Ser Leu Ala Arg Ala Gln Asp Ala Ile Ala Ser Gly 180 185 190	576
gcc ttc gcc gcc gag atc gcg ccc gtg acc gtc acg gca cgc aag gtg Ala Phe Ala Ala Glu Ile Ala Pro Val Thr Val Thr Ala Arg Lys Val 195 200 205	624
cag acc acc gtc gat acc gac gag atg ccc ggc aag gcc cgc ccc gag Gln Thr Thr Val Asp Thr Asp Glu Met Pro Gly Lys Ala Arg Pro Glu 210 215 220	672
aag atc ccc cat ctg aag ccc gcc ttc cgt gac ggt ggc acg gtc acg Lys Ile Pro His Leu Lys Pro Ala Phe Arg Asp Gly Gly Thr Val Thr 225 230 235 240	720
gcg gcg aac agc tcg tcg atc tcg gac ggg gcg gcg gcg ctg gtg atg Ala Ala Asn Ser Ser Ser Ile Ser Asp Gly Ala Ala Ala Leu Val Met 245 250 255	768
atg cgc cag tcg cag gcc gag aag ctg ggc ctg acg ccg atc gcg ccg Met Arg Gln Ser Gln Ala Glu Lys Leu Gly Leu Thr Pro Ile Ala Arg 260 265 270	816

atc atc ggt cat gcg acc cat gcc gac cgt ccc ggc ctg ttc cgc acg 864  
 Ile Ile Gly His Ala Thr His Ala Asp Arg Pro Gly Leu Phe Pro Thr  
 275 280 285

gcc ccc atc ggc gcg atg cgc aag ctg ctg gac cgc acg gac acc cgc 912  
 Ala Pro Ile Gly Ala Met Arg Lys Leu Leu Asp Arg Thr Asp Thr Arg  
 290 295 300

ctt ggc gat tac gac ctg ttc gag gtg aac gag gca ttc gcc gtc gtc 960  
 Leu Gly Asp Tyr Asp Leu Phe Glu Val Asn Glu Ala Phe Ala Val Val  
 305 310 315 320

gcc atg atc ggc atg aag gag ctt gcc ctg cca cac gat gcc acg aac 1008  
 Ala Met Ile Ala Met Lys Glu Leu Gly Leu Pro His Asp Ala Thr Asn  
 325 330 335

atc aac ggc ggg gcc tgc gcg ctt ggg cat ccc atc ggc gcg tgc ggg 1056  
 Ile Asn Gly Gly Ala Cys Ala Leu Gly His Pro Ile Gly Ala Ser Gly  
 340 345 350

gcg cgg atc atg gtc acg ctg ctg aac gcg atg gcg gcg cgg gcc gcg 1104  
 Ala Arg Ile Met Val Thr Leu Leu Asn Ala Met Ala Ala Arg Gly Ala  
 355 360 365

acg cgc ggg gcc gca tcc gtc tgc atc ggc ggg ggc gag gcg acg gcc 1152  
 Thr Arg Gly Ala Ala Ser Val Cys Ile Gly Gly Gly Glu Ala Thr Ala  
 370 375 380

atc gcg ctg gaa cgg ctg agc taa 1176  
 Ile Ala Leu Glu Arg Leu Ser  
 385 390

<210> 176

<211> 391

<212> PRT

<213> Paracoccus sp. R1534

<400> 176

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Phe Gln Gly Asp Leu Ala Ala Met Asp Ala Pro Thr Leu Gly Ala Ala  
 20 25 30

Ala Ile Arg Ala Ala Leu Asn Gly Leu Ser Pro Asp Met Val Asp Glu  
 35 40 45

Val Leu Met Gly Cys Val Leu Pro Ala Gly Gln Gly Gln Ala Pro Ala  
 50 55 60

Arg Gln Ala Ala Leu Asp Ala Gly Leu Pro Leu Ser Ala Gly Ala Thr  
 65 70 75 80

Thr Ile Asn Lys Met Cys Gly Ser Gly Met Lys Ala Ala Met Leu Gly  
 85 90 95

His Asp Leu Ile Ala Ala Gly Ser Ala Gly Ile Val Val Ala Gly Gly  
 100 105 110

Met Glu Ser Met Ser Asn Ala Pro Tyr Leu Leu Pro Lys Ala Arg Ser  
 115 120 125

Gly Met Arg Met Gly His Asp Arg Val Leu Asp His Met Phe Leu Asp  
 130 135 140

Gly Leu Glu Asp Ala Tyr Asp Lys Gly Arg Leu Met Gly Thr Phe Ala  
 145 150 155 160

Glu Asp Cys Ala Gly Asp His Gly Phe Thr Arg Glu Ala Gln Asp Asp  
 165 170 175

Tyr Ala Leu Thr Ser Leu Ala Arg Ala Gln Asp Ala Ile Ala Ser Gly  
 180 185 190

Ala Phe Ala Ala Glu Ile Ala Pro Val Thr Val Thr Ala Arg Lys Val  
 195 200 205

Gln Thr Thr Val Asp Thr Asp Glu Met Pro Gly Lys Ala Arg Pro Glu  
 210 215 220

Lys Ile Pro His Leu Lys Pro Ala Phe Arg Asp Gly Gly Thr Val Thr  
 225 230 235 240

Ala Ala Asn Ser Ser Ser Ile Ser Asp Gly Ala Ala Ala Leu Val Met  
 245 250 255

Met Arg Gln Ser Gln Ala Glu Lys Leu Gly Leu Thr Pro Ile Ala Arg  
 260 265 270

Ile Ile Gly His Ala Thr His Ala Asp Arg Pro Gly Leu Phe Pro Thr

275                      280                      285  
 Ala Pro Ile Gly Ala Met Arg Lys Leu Leu Asp Arg Thr Asp Thr Arg  
     290                      295                      300  
 Leu Gly Asp Tyr Asp Leu Phe Glu Val Asn Glu Ala Phe Ala Val Val  
     305                      310                      315                      320  
 Ala Met Ile Ala Met Lys Glu Leu Gly Leu Pro His Asp Ala Thr Asn  
                     325                      330                      335  
 Ile Asn Gly Gly Ala Cys Ala Leu Gly His Pro Ile Gly Ala Ser Gly  
                     340                      345                      350  
 Ala Arg Ile Met Val Thr Leu Leu Asn Ala Met Ala Ala Arg Gly Ala  
                     355                      360                      365  
 Thr Arg Gly Ala Ala Ser Val Cys Ile Gly Gly Gly Glu Ala Thr Ala  
                     370                      375                      380  
 Ile Ala Leu Glu Arg Leu Ser  
     385                      390  
  
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 <223> phaA  
  
 <220>  
 <221> misc\_feature  
 <222> (1179)..(1194)

<223> inverted repeat between genes constituting a putative transcript  
onal sto

<220>

<221> misc\_feature

<222> (1196)..(1210)

<223> inverted repeat between genes constituting a putative transcript  
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<221> CDS

<222> (1258)..(1980)

<223> phaB

<400> 177

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1 5 10 15	
ttc atg ggc gca ttc gcc aat gtc ccc gca cat gat ctg ggc gcc gcc	96
Phe Met Gly Ala Phe Ala Asn Val Pro Ala His Asp Leu Gly Ala Ala	
20 25 30	
gtc ctg cgc gag gtc gtg gcc cgc gcc ggt gtc gac ccc gcc gag gtc	144
Val Leu Arg Glu Val Val Ala Arg Ala Gly Val Asp Pro Ala Glu Val	
35 40 45	
agc gag acg atc ctg ggc cag gtg ctg acc gcc gcg cag ggc cag aac	192
Ser Glu Thr Ile Leu Gly Gln Val Leu Thr Ala Ala Gln Gly Gln Asn	
50 55 60	
ccc gcg cgc cag gcg cat atc aat acg gcc ctg ccc aag gaa tcg gcg	240
Pro Ala Arg Gln Ala His Ile Asn Ala Gly Leu Pro Lys Glu Ser Ala	
65 70 75 80	
gcg tgg ctc atc aac cag gtc tgc gcc tcg ggg ctg cgc gcc gtc gcg	288
Ala Trp Leu Ile Asn Gln Val Cys Gly Ser Gly Leu Arg Ala Val Ala	
85 90 95	
ctg gcg gcg cag cag gtc atg ctg gcc gat gcg cag atc gtt ctg gcg	336
Leu Ala Ala Gln Gln Val Met Leu Gly Asp Ala Gln Ile Val Leu Ala	
100 105 110	
ggg gcc cag gag agc atg tcg ctg tcg acc cat gcc gcc tat ctg cgc	384
Gly Gly Gln Glu Ser Met Ser Leu Ser Thr His Ala Ala Tyr Leu Arg	

115	120	125	
gcg ggc cag aag atg ggc Ala Gly Gln Lys Met Gly 130	gac atg aag atg atc Gly Asp Met Lys Met 135	gac acc atg atc cgc Asp Thr Met Ile Arg 140	432
gac ggg ctg tgg gat gcc Asp Gly Leu Trp Asp 145	ttc aac ggc tat cac Phe Asn Gly Tyr 150	atg ggt cag acc gcc His Met Gly Gln Thr Ala 155 160	480
gag aac gtg gcc gac Glu Asn Val Ala Asp 165	cag tgg tcg atc agc Gln Trp Ser Ile 170	cgc cag cag gac gaa Arg Asp Gln Gln Asp Glu 175	528
ttc gcc ctg gct tcg Phe Ala Leu Ala Ser 180	cag aac aag gcc gag Gln Asn Lys Ala Glu 185	gac ggc cgc cag aat Ala Ala Gln Asn Ala Gly 190	576
cgc ttc gat gac gaa atc Arg Phe Asp Asp Glu 195	gtc gcc tat acc gtc Val Ala Tyr Thr 200	aag ggc cgc aag ggc Lys Gly Arg Lys Gly 205	624
gac acg gtc gtc gac Asp Thr Val Val Asp 210	aag gac gaa tac atc Lys Asp Glu Tyr Ile 215	cgc cac ggc gcc acg atc Arg His Gly Ala Thr Ile 220	672
gag ggc atg cag aag Glu Gly Met Gln Lys 225	ctg cgc ccc gcc ttc Leu Arg Pro Ala Phe 230	acc aag gaa ggc tgc Thr Lys Glu Gly Ser Val 235 240	720
acg cgc ggc aac gcg Thr Ala Gly Asn Ala 245	tcg ggc ctg aac gac Ser Gly Leu Asn Asp 250	ggc cgc gcg gcc gtc atg Gly Ala Ala Ala Val Met 255	768
gtc atg tcc gag gac Val Met Ser Glu Asp 260	gag gcc gca cgc cgc Glu Ala Ala Arg Arg 265	ctg acg ccg ctg gcg Leu Thr Pro Leu Ala 270	816
cgc atc gcc tcc tat Arg Ile Ala Ser Tyr 275	gcg acg gcg ggc ctc Ala Thr Ala Gly Leu 280	gac ccg gcg atc atg ggc Asp Pro Ala Ile Met Gly 285	864
acc ggg ccg atc ccc Thr Gly Pro Ile Pro 290	tcc agc cgc aag gcg Ser Ser Arg Lys Ala 295	ctg gaa aag gcg ggc tgg Leu Glu Lys Ala Gly Trp 300	912
tcg gtc ggc gac ctg Ser Val Gly Asp Leu 305	gac ctg gtc gag gcg Asp Leu Val Glu Ala 310	aac gag gcc ttt gcc gcg Asn Glu Ala Phe Ala Ala 315 320	960
cag gcc tgc gcc gtg Gln Ala Cys Ala Val 325	aac aag gac atg ggc Asn Lys Asp Met Gly 330	tggtgat ccg tcc atc gtg Trp Asp Pro Ser Ile Val 335	1008
aac gtc aac ggc ggc Asn Val Asn Gly Gly 340	gcg atc gcc atc ggc Ala Ile Ala Ile Gly 345	cac ccg atc ggc gcc tgc His Pro Ile Gly Ala Ser 350	1056



ggg gcg cgg atc ctg aac acc ctg ctg ttc gag atg cag cgc cgc gac Gly Ala Arg Ile Leu Asn Thr Leu Leu Phe Glu Met Gln Arg Arg Asp 355 360 365	1104
gcc aag aag ggc ctt gcg acg ctg tgc atc ggc ggc ggc atg ggc gtc Ala Lys Lys Gly Leu Ala Thr Leu Cys Ile Gly Gly Gly Met Gly Val 370 375 380	1152
gcc atg tgc ctc gaa cgc tgaacgaccg gcgtgtgcgc aatttaattg Ala Met Cys Leu Glu Arg 385 390	1200
cgcacacgcc ccctgcaaaag tagcaatggt ttacgataac gaatagaagg gggaaac	1257
atg tcc aag gta gca ctg gtc acc ggc gga tgc cgc ggc atc ggc gcc Met Ser Lys Val Ala Leu Val Thr Gly Gly Ser Arg Gly Ile Gly Ala 395 400 405	1305
gag atc tgc aag gcg ctt cag gcc gca ggc tat acc gtc gcc gcg aac Glu Ile Cys Lys Lys Ala Leu Gln Ala Ala Gly Tyr Thr Val Ala Ala Asn 410 415 420	1353
tat gcc ggc aat gac gac gcg gcc aag gcc ttc acc gag gaa acc ggc Tyr Ala Gly Asn Asp Asp Ala Ala Lys Ala Phe Thr Glu Glu Thr Gly 425 430 435	1401
atc aag acc tac aag tgg tgc gtc gcc gat tac gat gcc tgc aag gcc Ile Lys Thr Tyr Lys Trp Ser Val Ala Asp Tyr Asp Ala Cys Lys Ala 440 445 450	1449
ggc atc gcc cag gtc gaa gag gat ctg ggc ccg atc gcc gtg ctg atc Gly Ile Ala Gln Val Glu Glu Asp Leu Gly Pro Ile Ala Val Leu Ile 455 460 465 470	1497
aac aat gcc ggg atc acc cgc gac gcg ccc ttc cac aag atg acg ccc Asn Asn Ala Gly Ile Thr Arg Asp Ala Pro Phe His Lys Met Thr Pro 475 480 485	1545
gag aag tgg aag gag gtc atc gac acc aac ctg acc ggc acc ttc aac Glu Lys Trp Lys Glu Val Ile Asp Thr Asn Leu Thr Gly Thr Phe Asn 490 495 500	1593
atg acc cat cgc gtc tgg cgc ggc atg cgc gaa cgc aag ttc gga cgc Met Thr His Pro Val Trp Pro Gly Met Arg Glu Arg Lys Phe Gly Arg 505 510 515	1641
gtc atc aac atc agc tgc atc aac ggg cag aag ggc cag ttc ggg cag Val Ile Asn Ile Ser Ser Ile Asn Gly Gln Lys Gly Gln Phe Gly Gln 520 525 530	1689
gcg aac tat gcc gcg gcc aag gcg ggc gac ctg ggc ttc acc aag tgc Ala Asn Tyr Ala Ala Lys Ala Gly Asp Leu Gly Phe Thr Lys Ser 535 540 545 550	1737
ctg gcg cag gaa ggc gcg cgc aac aac atc acc gtc aac gcg atc tgc Leu Ala Gln Glu Gly Ala Arg Asn Asn Ile Thr Val Asn Ala Ile Cys	1785

	555	560	565	
ccc ggc tat atc gcg acg gac atg gtg atg gcc gtt ccc gaa cag gtc				1833
Pro Gly Tyr Ile Ala Thr Asp Met Val Met Ala Val Pro Glu Gln Val				
	570	575	580	
cgc gag ggg atc atc gcg cag atc ccc gtc ggc cgc ttg ggc gag cgc				1881
Arg Glu Gly Ile Ile Ala Gln Ile Pro Val Gly Arg Leu Gly Glu Pro				
	585	590	595	
tcc gag atc gcg cgc tgc gtg gtg ttc ctg gcc tcc gac gat cgc ggc				1929
Ser Glu Ile Ala Arg Cys Val Val Phe Leu Ala Ser Asp Asp Ala Gly				
	600	605	610	
ttc gtc aca ggc tcg acc atc acg gcg aat ggc ggc cag tac tac atc				1977
Phe Val Thr Gly Ser Thr Ile Thr Ala Asn Gly Gly Gln Tyr Tyr Ile				
	615	620	625	630
tga				1980
<210>	178			
<211>	390			
<212>	PRT			
<213>	Paracoccus sp. R114			
<220>				
<221>	misc_feature			
<222>	(1179)..(1194)			
<223>	inverted repeat between genes constituting a putative transcripti onal sto			
<220>				
<221>	misc_feature			
<222>	(1196)..(1210)			
<223>	inverted repeat between genes constituting a putative transcripti onal sto			
<400>	178			
Met Thr Lys Ala Val Ile Val Ser Ala Ala Arg Thr Pro Val Gly Ser				
1	5	10	15	
Phe Met Gly Ala Phe Ala Asn Val Pro Ala His Asp Leu Gly Ala Ala				
	20	25	30	

Val Leu Arg Glu Val Val Ala Arg Ala Gly Val Asp Pro Ala Glu Val  
 35 40 45

Ser Glu Thr Ile Leu Gly Gln Val Leu Thr Ala Ala Gln Gly Gln Asn  
 50 55 60

Pro Ala Arg Gln Ala His Ile Asn Ala Gly Leu Pro Lys Glu Ser Ala  
 65 70 75 80

Ala Trp Leu Ile Asn Gln Val Cys Gly Ser Gly Leu Arg Ala Val Ala  
 85 90 95

Leu Ala Ala Gln Gln Val Met Leu Gly Asp Ala Gln Ile Val Leu Ala  
 100 105 110

Gly Gly Gln Glu Ser Met Ser Leu Ser Thr His Ala Ala Tyr Leu Arg  
 115 120 125

Ala Gly Gln Lys Met Gly Asp Met Lys Met Ile Asp Thr Met Ile Arg  
 130 135 140

Asp Gly Leu Trp Asp Ala Phe Asn Gly Tyr His Met Gly Gln Thr Ala  
 145 150 155 160

Glu Asn Val Ala Asp Gln Trp Ser Ile Ser Arg Asp Gln Gln Asp Glu  
 165 170 175

Phe Ala Leu Ala Ser Gln Asn Lys Ala Glu Ala Ala Gln Asn Ala Gly  
 180 185 190

Arg Phe Asp Asp Glu Ile Val Ala Tyr Thr Val Lys Gly Arg Lys Gly  
 195 200 205

Asp Thr Val Val Asp Lys Asp Glu Tyr Ile Arg His Gly Ala Thr Ile  
 210 215 220

Glu Gly Met Gln Lys Leu Arg Pro Ala Phe Thr Lys Glu Gly Ser Val  
 225 230 235 240

Thr Ala Gly Asn Ala Ser Gly Leu Asn Asp Gly Ala Ala Ala Val Met  
 245 250 255

Val Met Ser Glu Asp Glu Ala Ala Arg Arg Gly Leu Thr Pro Leu Ala  
 260 265 270

Arg Ile Ala Ser Tyr Ala Thr Ala Gly Leu Asp Pro Ala Ile Met Gly  
 275 280 285

Thr Gly Pro Ile Pro Ser Ser Arg Lys Ala Leu Glu Lys Ala Gly Trp  
 290 295 300

Ser Val Gly Asp Leu Asp Leu Val Glu Ala Asn Glu Ala Phe Ala Ala  
 305 310 315 320

Gln Ala Cys Ala Val Asn Lys Asp Met Gly Trp Asp Pro Ser Ile Val  
 325 330 335

Asn Val Asn Gly Gly Ala Ile Ala Ile Gly His Pro Ile Gly Ala Ser  
 340 345 350

Gly Ala Arg Ile Leu Asn Thr Leu Leu Phe Glu Met Gln Arg Arg Asp  
 355 360 365

Ala Lys Lys Gly Leu Ala Thr Leu Cys Ile Gly Gly Gly Met Gly Val  
 370 375 380

Ala Met Cys Leu Glu Arg  
 385 390

<210> 179

<211> 240

<212> PRT

<213> *Paracoccus* sp. R114

<220>

<221> misc\_feature

<222> (1179)..(1194)

<223> inverted repeat between genes constituting a putative transcripti  
 onal sto

<220>

<221> misc\_feature

<222> (1196)..(1210)

<223> inverted repeat between genes constituting a putative transcript  
onal sto

<400> 179

Met Ser Lys Val Ala Leu Val Thr Gly Gly Ser Arg Gly Ile Gly Ala  
1 5 10 15

Glu Ile Cys Lys Ala Leu Gln Ala Ala Gly Tyr Thr Val Ala Ala Asn  
20 25 30

Tyr Ala Gly Asn Asp Asp Ala Ala Lys Ala Phe Thr Glu Glu Thr Gly  
35 40 45

Ile Lys Thr Tyr Lys Trp Ser Val Ala Asp Tyr Asp Ala Cys Lys Ala  
50 55 60

Gly Ile Ala Gln Val Glu Glu Asp Leu Gly Pro Ile Ala Val Leu Ile  
65 70 75 80

Asn Asn Ala Gly Ile Thr Arg Asp Ala Pro Phe His Lys Met Thr Pro  
85 90 95

Glu Lys Trp Lys Glu Val Ile Asp Thr Asn Leu Thr Gly Thr Phe Asn  
100 105 110

Met Thr His Pro Val Trp Pro Gly Met Arg Glu Arg Lys Phe Gly Arg  
115 120 125

Val Ile Asn Ile Ser Ser Ile Asn Gly Gln Lys Gly Gln Phe Gly Gln  
130 135 140

Ala Asn Tyr Ala Ala Ala Lys Ala Gly Asp Leu Gly Phe Thr Lys Ser  
145 150 155 160

Leu Ala Gln Glu Gly Ala Arg Asn Asn Ile Thr Val Asn Ala Ile Cys  
165 170 175

Pro Gly Tyr Ile Ala Thr Asp Met Val Met Ala Val Pro Glu Gln Val  
180 185 190

Arg Glu Gly Ile Ile Ala Gln Ile Pro Val Gly Arg Leu Gly Glu Pro  
 195 200 205

Ser Glu Ile Ala Arg Cys Val Val Phe Leu Ala Ser Asp Asp Ala Gly  
 210 215 220

Phe Val Thr Gly Ser Thr Ile Thr Ala Asn Gly Gly Gln Tyr Tyr Ile  
 225 230 235 240

<210> 180

<211> 729

<212> DNA

<213> *Paracoccus carotinifaciens* E-396

<220>

<221> CDS

<222> (1)..(726)

<223> Beta-carotene Beta-4 oxygenase

<400> 180  
 atg agc gca cat gcc ctg ccc aag gca gat ctg acc gcc acc agt ttg 48  
 Met Ser Ala His Ala Leu Pro Lys Ala Asp Leu Thr Ala Thr Ser Leu  
 1 5 10 15  
 atc gtc tcg ggc ggc atc atc gcc gcg tgg ctg gcc ctg cat gtg cat 96  
 Ile Val Ser Gly Gly Ile Ile Ala Ala Trp Leu Ala Leu His Val His  
 20 25 30  
 gcg ctg tgg ttt ctg gac gcg gcg gcg cat ccc atc ctg gcg gtc gcg 144  
 Ala Leu Trp Phe Leu Asp Ala Ala Ala His Pro Ile Leu Ala Val Ala  
 35 40 45  
 aat ttc ctg ggg ctg acc tgg ctg tcg gtc ggt ctg ttc atc atc gcg 192  
 Asn Phe Leu Gly Leu Thr Trp Leu Ser Val Gly Leu Phe Ile Ile Ala  
 50 55 60  
 cat gac gcg atg cat ggg tcg gtc gtg ccg ggg cgc ccg cgc gcc aat 240  
 His Asp Ala Met His Gly Ser Val Val Pro Gly Arg Pro Arg Ala Asn  
 65 70 75 80  
 gcg gcg atg ggc cag ctt gtc ctg tgg ctg tat gcc gga ttt tcc tgg 288  
 Ala Ala Met Gly Gln Leu Val Leu Trp Leu Tyr Ala Gly Phe Ser Trp  
 85 90 95

cgc aag atg atc gtc aag cac atg gcc cat cat cgc cat gcc gga acc 336  
 Arg Lys Met Ile Val Lys His Met Ala His His Arg His Ala Gly Thr  
 100 105 110  
 gac gac gac cca gat ttc gac cat ggc ggc ccg gtc cgc tgg tac gcc 384  
 Asp Asp Asp Pro Asp Phe Asp His Gly Gly Pro Val Arg Trp Tyr Ala  
 115 120 125  
 cgc ttc atc ggc acc tat ttc ggc tgg cgc gag ggg ctg ctg ctg ccc 432  
 Arg Phe Ile Gly Thr Tyr Phe Gly Trp Arg Glu Gly Leu Leu Leu Pro  
 130 135 140  
 gtc atc gtg acg gtc tat gcg ctg atg ttg ggg gat cgc tgg atg tac 480  
 Val Ile Val Thr Val Tyr Ala Leu Met Leu Gly Asp Arg Trp Met Tyr  
 145 150 155 160  
 gtg gtc ttc tgg ccg ttg ccg tcg atc ctg gcg tcg atc cag ctg ttc 528  
 Val Val Phe Trp Pro Leu Pro Ser Ile Leu Ala Ser Ile Gln Leu Phe  
 165 170 175  
 gtg ttc ggc atc tgg ctg ccg cac cgc ccc ggc cac gac gcg ttc ccg 576  
 Val Phe Gly Ile Trp Leu Pro His Arg Pro Gly His Asp Ala Phe Pro  
 180 185 190  
 gac cgc cac aat gcg ccg tcg tcg ccg atc agc gac ccc gtg tcg ctg 624  
 Asp Arg His Asn Ala Arg Ser Ser Arg Ile Ser Asp Pro Val Ser Leu  
 195 200 205  
 ctg acc tgc ttt cac ttt ggc ggt tat cat cac gaa cac cac ctg cac 672  
 Leu Thr Cys Phe His Phe Gly Gly Tyr His His Glu His His Leu His  
 210 215 220  
 ccg acg gtg cct tgg tgg ccg ctg ccc agc acc cgc acc aag ggg gac 720  
 Pro Thr Val Pro Trp Trp Arg Leu Pro Ser Thr Arg Thr Lys Gly Asp  
 225 230 235 240  
 acc gca tga 729  
 Thr Ala

&lt;210&gt; 181

&lt;211&gt; 242

&lt;212&gt; PRT

<213> *Paracoccus carotinifaciens* E-396

&lt;400&gt; 181

Met Ser Ala His Ala Leu Pro Lys Ala Asp Leu Thr Ala Thr Ser Leu  
 1 5 10 15

Ile Val Ser Gly Gly Ile Ile Ala Ala Trp Leu Ala Leu His Val His  
 20 25 30  
 Ala Leu Trp Phe Leu Asp Ala Ala Ala His Pro Ile Leu Ala Val Ala  
 35 40 45  
 Asn Phe Leu Gly Leu Thr Trp Leu Ser Val Gly Leu Phe Ile Ile Ala  
 50 55 60  
 His Asp Ala Met His Gly Ser Val Val Pro Gly Arg Pro Arg Ala Asn  
 65 70 75 80  
 Ala Ala Met Gly Gln Leu Val Leu Trp Leu Tyr Ala Gly Phe Ser Trp  
 85 90 95  
 Arg Lys Met Ile Val Lys His Met Ala His His Arg His Ala Gly Thr  
 100 105 110  
 Asp Asp Asp Pro Asp Phe Asp His Gly Gly Pro Val Arg Trp Tyr Ala  
 115 120 125  
 Arg Phe Ile Gly Thr Tyr Phe Gly Trp Arg Glu Gly Leu Leu Leu Pro  
 130 135 140  
 Val Ile Val Thr Val Tyr Ala Leu Met Leu Gly Asp Arg Trp Met Tyr  
 145 150 155 160  
 Val Val Phe Trp Pro Leu Pro Ser Ile Leu Ala Ser Ile Gln Leu Phe  
 165 170 175  
 Val Phe Gly Ile Trp Leu Pro His Arg Pro Gly His Asp Ala Phe Pro  
 180 185 190  
 Asp Arg His Asn Ala Arg Ser Ser Arg Ile Ser Asp Pro Val Ser Leu  
 195 200 205  
 Leu Thr Cys Phe His Phe Gly Gly Tyr His His Glu His His Leu His  
 210 215 220  
 Pro Thr Val Pro Trp Trp Arg Leu Pro Ser Thr Arg Thr Lys Gly Asp  
 225 230 235 240



Thr Ala

&lt;210&gt; 182

&lt;211&gt; 510

&lt;212&gt; DNA

&lt;213&gt; Paracoccus sp. R1534

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(507)

&lt;223&gt; Beta-Carotene hydroxylase

&lt;400&gt; 182

atg	agc	act	tgg	gcc	gca	atc	ctg	acc	gtc	atc	ctg	acc	gtc	gcc	gcg	48
Met	Ser	Thr	Trp	Ala	Ala	Ile	Leu	Thr	Val	Ile	Leu	Thr	Val	Ala	Ala	
1				5					10					15		

atg	gag	ctg	acg	gcc	tac	tcc	gtc	cat	cgg	tgg	atc	atg	cat	ggc	ccc	96
Met	Glu	Leu	Thr	Ala	Tyr	Ser	Val	His	Arg	Trp	Ile	Met	His	Gly	Pro	
			20					25						30		

ctg	ggc	tgg	ggc	tgg	cat	aaa	tcg	cac	cac	gac	gag	gat	cac	gac	cac	144
Leu	Gly	Trp	Gly	Trp	His	Lys	Ser	His	His	Asp	Glu	Asp	His	Asp	His	
		35					40					45				

gcg	ctc	gag	aag	aac	gac	ctc	tat	ggc	gtc	atc	ttc	gcg	gta	atc	tcg	192
Ala	Leu	Glu	Lys	Asn	Asp	Leu	Tyr	Gly	Val	Ile	Phe	Ala	Val	Ile	Ser	
	50					55					60					

atc	gtg	ctg	ttc	gcg	atc	ggc	gcg	atg	ggg	tcg	gat	ctg	gcc	tgg	tgg	240
Ile	Val	Leu	Phe	Ala	Ile	Gly	Ala	Met	Gly	Ser	Asp	Leu	Ala	Trp	Trp	
65					70				75					80		

ctg	gcg	gtg	ggg	gtc	acc	tgc	tac	ggg	ctg	atc	tac	tat	ttc	ctg	cat	288
Leu	Ala	Val	Gly	Val	Thr	Cys	Tyr	Gly	Leu	Ile	Tyr	Tyr	Phe	Leu	His	
				85					90					95		

gac	ggc	ttg	gtg	cat	ggg	cgc	tgg	cgg	ttc	cgc	tat	gtc	ccc	aag	cgc	336
Asp	Gly	Leu	Val	His	Gly	Arg	Trp	Pro	Phe	Arg	Tyr	Val	Pro	Lys	Arg	
			100						105				110			

ggc	tat	ctt	cgt	cgc	gtc	tac	cag	gca	cac	agg	atg	cat	cac	gcg	gtc	384
Gly	Tyr	Leu	Arg	Arg	Val	Tyr	Gln	Ala	His	Arg	Met	His	His	Ala	Val	
		115					120							125		

cat ggc cgc gag aac tgc gtc agc ttc ggt ttc atc tgg gcg ccc tcg 432  
 His Gly Arg Glu Asn Cys Val Ser Phe Gly Phe Ile Trp Ala Pro Ser  
 130 135 140

gtc gac agc ctc aag gca gag ctg aaa cgc tgc ggc gcg ctg ctg aag 480  
 Val Asp Ser Leu Lys Ala Glu Leu Lys Arg Ser Gly Ala Leu Leu Lys  
 145 150 155 160

gac cgc gaa ggg gcg gat cgc aat aca tga 510  
 Asp Arg Glu Gly Ala Asp Arg Asn Thr  
 165

<210> 183

<211> 169

<212> PRT

<213> Paracoccus sp. R1534

<400> 183

Met Ser Thr Trp Ala Ala Ile Leu Thr Val Ile Leu Thr Val Ala Ala  
 1 5 10 15

Met Glu Leu Thr Ala Tyr Ser Val His Arg Trp Ile Met His Gly Pro  
 20 25 30

Leu Gly Trp Gly Trp His Lys Ser His His Asp Glu Asp His Asp His  
 35 40 45

Ala Leu Glu Lys Asn Asp Leu Tyr Gly Val Ile Phe Ala Val Ile Ser  
 50 55 60

Ile Val Leu Phe Ala Ile Gly Ala Met Gly Ser Asp Leu Ala Trp Trp  
 65 70 75 80

Leu Ala Val Gly Val Thr Cys Tyr Gly Leu Ile Tyr Tyr Phe Leu His  
 85 90 95

Asp Gly Leu Val His Gly Arg Trp Pro Phe Arg Tyr Val Pro Lys Arg  
 100 105 110

Gly Tyr Leu Arg Arg Val Tyr Gln Ala His Arg Met His His Ala Val  
 115 120 125

His Gly Arg Glu Asn Cys Val Ser Phe Gly Phe Ile Trp Ala Pro Ser  
130 135 140

Val Asp Ser Leu Lys Ala Glu Leu Lys Arg Ser Gly Ala Leu Leu Lys  
145 150 155 160

Asp Arg Glu Gly Ala Asp Arg Asn Thr  
165

<210> 184

<211> 888

<212> DNA

<213> *Paracoccus* sp. R1534

<220>

<221> CDS

<222> (1)..(885)

<223> farnesyltransferase or geranylgeranyl diphosphate synthase

<400> 184  
atg acg ccc aag cag caa ttc ccc cta cgc gat ctg gtc gag atc agg 48  
Met Thr Pro Lys Gln Gln Phe Pro Leu Arg Asp Leu Val Glu Ile Arg  
1 5 10 15  
ctg gcg cag atc tcg gcc cag ttc gcc gtg gtc tcg gcc cgc ctg gcc 96  
Leu Ala Gln Ile Ser Gly Gln Phe Gly Val Val Ser Ala Pro Leu Gly  
20 25 30  
gcg gcc atg agc gat gcc gcc ctg tcc ccc gcc aaa cgc ttt cgc gcc 144  
Ala Ala Met Ser Asp Ala Ala Leu Ser Pro Gly Lys Arg Phe Arg Ala  
35 40 45  
gtg ctg atg ctg atg gtc gcc gaa agc tcg gcc ggg gtc tgc gat gcg 192  
Val Leu Met Leu Met Val Ala Glu Ser Ser Gly Gly Val Cys Asp Ala  
50 55 60  
atg gtc gat gcc gcc tgc gcg gtc gag atg gtc cat gcc gca tcg ctg 240  
Met Val Asp Ala Ala Cys Ala Val Glu Met Val His Ala Ala Ser Leu  
65 70 75 80  
atc ttc gac gac atg ccc tgc atg gac gat gcc agg acc cgt cgc ggt 288  
Ile Phe Asp Asp Met Pro Cys Met Asp Asp Ala Arg Thr Arg Arg Gly  
85 90 95

cag ccc gcc acc cat gtc gcc cat ggc gag ggc cgc gcg gtg ctt gcg 336  
 Gln Pro Ala Thr His Val Ala His Gly Glu Gly Arg Ala Val Leu Ala  
 100 105 110

ggc atc gcc ctg atc acc gag gcc atg cgg att ttg ggc gag gcg cgc 384  
 Gly Ile Ala Leu Ile Thr Glu Ala Met Arg Ile Leu Gly Glu Ala Arg  
 115 120 125

ggc gcg acg ccg gat cag cgc gca agg ctg gtc gca tcc atg tcg cgc 432  
 Gly Ala Thr Pro Asp Gln Arg Ala Arg Leu Val Ala Ser Met Ser Arg  
 130 135 140

gcg atg gga ccg gtg ggg ctg tgc gca ggc cag gat ctg gac ctg cac 480  
 Ala Met Gly Pro Val Gly Leu Cys Ala Gly Gln Asp Leu Asp Leu His  
 145 150 155 160

gcc ccc aag gac gcc gcc ggg atc gaa cgt gaa cag gac ctc aag acc 528  
 Ala Pro Lys Asp Ala Ala Gly Ile Glu Glu Gln Asp Leu Lys Thr  
 165 170 175

ggc gtg ctg ttc gtc gcg gcc ctc gag atg ctg tcc att att aag ggt 576  
 Gly Val Leu Phe Val Ala Gly Leu Glu Met Leu Ser Ile Ile Lys Gly  
 180 185 190

ctg gac aag gcc gag acc gag cag ctc atg gcc ttc ggc cgt cag ctt 624  
 Leu Asp Lys Ala Glu Thr Glu Gln Leu Met Ala Phe Gly Arg Gln Leu  
 195 200 205

ggt cgg gtc ttc cag tcc tat gac gac ctg ctg gac gtg atc ggc gac 672  
 Gly Arg Val Phe Gln Ser Tyr Asp Asp Leu Leu Asp Val Ile Gly Asp  
 210 215 220

aag gcc agc acc ggc aag gat acg ggc cgc gac acc gcc gcc ccc ggc 720  
 Lys Ala Ser Thr Gly Lys Asp Thr Gly Arg Asp Thr Ala Ala Pro Gly  
 225 230 235 240

cca aag cgc ggc ctg atg gcg gtc gga cag atg ggc gac gtg gcg cag 768  
 Pro Lys Arg Gly Leu Met Ala Val Gly Gln Met Gly Asp Val Ala Gln  
 245 250 255

cat tac cgc gcc agc cgc gcg caa ctg gac gag ctg atg cgc acc cgg 816  
 His Tyr Arg Ala Ser Arg Ala Gln Leu Asp Glu Leu Met Arg Thr Arg  
 260 265 270

ctg ttc cgc ggg ggg cag atc gcg gac ctg ctg gcc cgc gtg ctg ccg 864  
 Leu Phe Arg Gly Gly Gln Ile Ala Asp Leu Leu Ala Arg Val Leu Pro  
 275 280 285

cat gac atc cgc cgc agc gcc tag 888  
 His Asp Ile Arg Arg Ser Ala  
 290 295

&lt;210&gt; 185

&lt;211&gt; 295

&lt;212&gt; PRT

&lt;213&gt; Paracoccus sp. R1534

&lt;400&gt; 185

Met Thr Pro Lys Gln Gln Phe Pro Leu Arg Asp Leu Val Glu Ile Arg  
 1 5 10 15

Leu Ala Gln Ile Ser Gly Gln Phe Gly Val Val Ser Ala Pro Leu Gly  
 20 25 30

Ala Ala Met Ser Asp Ala Ala Leu Ser Pro Gly Lys Arg Phe Arg Ala  
 35 40 45

Val Leu Met Leu Met Val Ala Glu Ser Ser Gly Gly Val Cys Asp Ala  
 50 55 60

Met Val Asp Ala Ala Cys Ala Val Glu Met Val His Ala Ala Ser Leu  
 65 70 75 80

Ile Phe Asp Asp Met Pro Cys Met Asp Asp Ala Arg Thr Arg Arg Gly  
 85 90 95

Gln Pro Ala Thr His Val Ala His Gly Glu Gly Arg Ala Val Leu Ala  
 100 105 110

Gly Ile Ala Leu Ile Thr Glu Ala Met Arg Ile Leu Gly Glu Ala Arg  
 115 120 125

Gly Ala Thr Pro Asp Gln Arg Ala Arg Leu Val Ala Ser Met Ser Arg  
 130 135 140

Ala Met Gly Pro Val Gly Leu Cys Ala Gly Gln Asp Leu Asp Leu His  
 145 150 155 160

Ala Pro Lys Asp Ala Ala Gly Ile Glu Arg Glu Gln Asp Leu Lys Thr  
 165 170 175

Gly Val Leu Phe Val Ala Gly Leu Glu Met Leu Ser Ile Ile Lys Gly  
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Leu Asp Lys Ala Glu Thr Glu Gln Leu Met Ala Phe Gly Arg Gln Leu

195	200	205
Gly Arg Val Phe Gln Ser Tyr Asp Asp Leu Leu Asp Val Ile Gly Asp		
210	215	220
Lys Ala Ser Thr Gly Lys Asp Thr Gly Arg Asp Thr Ala Ala Pro Gly		
225	230	240
Pro Lys Arg Gly Leu Met Ala Val Gly Gln Met Gly Asp Val Ala Gln		
245	250	255
His Tyr Arg Ala Ser Arg Ala Gln Leu Asp Glu Leu Met Arg Thr Arg		
260	265	270
Leu Phe Arg Gly Gly Gln Ile Ala Asp Leu Leu Ala Arg Val Leu Pro		
275	280	285
His Asp Ile Arg Arg Ser Ala		
290	295	

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&lt;211&gt; 30

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52

# ATCC

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## BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

### INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3  
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Roche Vitamins Inc.  
Attn: Markus Huesbelin  
340 Kingsland Street  
Nutley, NJ 007110-1199

Deposited on Behalf of: Roche Vitamins Inc.

Identification Reference by Depositor:

Patent Deposit Designation

Paracoccus sp.: R-1506

PTA-3431

The deposit was accompanied by:     a scientific description     a proposed taxonomic description indicated above.

The deposit was received June 5, 2001 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: ☒ We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested June 27, 2001. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

  
Tanya Nunnally, Patent Specialist, Patent Depository

Date: June 28, 2001

cc: Kevin C. Hooper  
(Ref: Docket or Case No.: C38435/121966)

# ATCC

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## BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

### INTERNATIONAL FORM

#### RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Roche Vitamins Inc.  
Attn: Marlene Huenkelin  
340 Kingsland Street  
Nutley, NJ 07110-1199

Deposited on Behalf of: Roche Vitamins Inc.

Identification Reference by Depositor:

Paracoccus sp.: R114

Paracoccus sp.: R1534

Patent Deposit Designation

PTA-3335

PTA-3336

The deposits were accompanied by:    a scientific description, a proposed taxonomic description indicated above. The deposits were received April 24, 2001 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested May 7, 2001. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

  
Tanya Nunnally, Patent Specialist, Patent Depository

Date: May 23, 2001

cc: Kevin C. Hooper

(Ref: Docket or Case No.: C38435/121966)